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4425

Influence of Ultraviolet Light, Solar Irradiation and Cod-Liver Oil on Production, Fertility and Incubation of Eggs.*

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During the summers of 1926 and 1927 several sets of experiments were conducted in which eggs (from the same flock of white leghorns) were incubated under normal and under abnormal conditions. In all instances eggs were arranged in such a manner that some groups did not receive irradiation from a quartz-mercury arc whereas other groups were irradiated for 10, 15 or 30 minutes a day, respectively. In general, these experiments showed that the daily average loss of weight for each egg when normally incubated amounted to 0.35 gm. Under abnormal incubation (with excessive moisture in the incubator) the loss of weight when the eggs were not irradiated was about 0.20 gm. With normal incubation, irradiation with ultraviolet light shortened the period of incubation 24 to 48 hours in about 20% of the eggs. The percentage hatch of normally incubated irradiated and nonirradiated eggs was practically the same. With abnormal incubation, ultraviolet irradiation increased the daily loss of weight and, with 30 minutes of irradiation, normal loss of weight was produced. Chicks hatched with abnormally incubated eggs when irradiated 15 to 30 minutes a day were in general normal. Without irradiation, pendant or unabsorbed yolk sacs were much in evidence. The hatch under normal incubation without irradiation was, in one of the experiments, 75%, and with irradiation 70%, and under abnormal incubation, without irradiation, 13%, and with irradiation 30%. These experiments indicate that irradiation with quartz-mercury arcs tends to increase the rate of metabolism of the incubating eggs and that, under abnormal incubation and unphysiologic conditions, *ultraviolet irradiation is an accessory in maintaining normal metabolism.* This viewpoint is substantiated further by various other investigations we have made on the effects of radiant energy on the germination and growth of seeds and the changes in metabolic rates induced in plants by irradiation of suitable quantity and quality.

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* An attempt has not been made in this abstract to review the literature. Reference, however, should be made to the work of Hart, Steenbock, Lepkovsky, Kletzien, Halpin and Johnson, *J. Biol. Chem.*, 1925, lxv, 571-595 (and appended bibliography); Hughes, Titus and Moore, *Science*, 1925, lxii, 492-493, and Hughes, Titus and Smits, *Science*, 1927, lxv, 264.

Transmission of radiant energy and gases through eggshells.—Spectrograms obtained with a wide spectroscopic slit and with exposure of a photographic plate for 2 to 5 minutes indicate that the shell and membrane transmit ultraviolet light to about 300 millimicrons whereas the membrane alone transmits to about 270 millimicrons. Proof is furnished, therefore, from the spectroscopic standpoint that radiant energy of wavelengths known to produce biologic effects can be transmitted through the combined shell and lining membrane. From the results which we have obtained regarding the influence which irradiation from a quartz-mercury arc exerts on the development of abnormally incubated eggs and so forth, we may conclude that sufficient radiant energy of a stimulating character can be transmitted to the ovum of the incubating egg to act as an accessory factor in normal development when this development is taking place under abnormal or unphysiologic conditions.

One other possible source of metabolic significance should be mentioned, namely, the presence of ozone and oxides of nitrogen. These gases are produced by quartz-mercury arcs and are, in part at least, in an ionized condition. In the exchange of gases taking place between the air and the incubating egg, it is possible that these ionized or activated gases may pass into the egg and exert an influence on the metabolic processes within the egg. Such an hypothesis is substantiated in part by the marked effects produced in the potential differences present in leaves of plants which are supplied with gases sucked from the region of enclosed arc discharges as reported by Sheard and Johnson.

In other experiments, the shells of eggs were removed from an area of approximately 5 sq. cm., care being exercised to prevent puncture of the underlying membrane. The areas from which the shell had been removed were closed with microscopic cover slips of glass and of quartz respectively. These cover slips were attached so as to provide air-tight seals. Daily exposure of the ovum of normally incubated eggs to ultraviolet irradiation for 30 to 60 seconds through the glass cover slips did not produce changes in the development or growth of the embryos. When exactly similar experiments were carried out through quartz cover slips, various types of abnormal development were produced. These will be described in detail in the completed paper. These results show that there is sufficient transmission of ultraviolet radiation by quartz (over and above that transmitted by equal thicknesses of glass) and the membrane lining the shell to produce marked changes in embryonic development.

Effects of solar irradiation and cod-liver oil on production and fertility of eggs.—Three groups of 5 hens and cockerel (all from the same good quality stock which had been out of doors all summer) were housed in separate compartments in an experimental hen house in the autumn of 1927. All groups were fed the standard Wisconsin ration containing full quota of all food constituents and vitamines except vitamine D. Group 1 was enclosed behind a large window of quartz-containing glass (vitaglass), group 2 behind ordinary window glass and group 3 behind ordinary window glass, with 2% by weight of cod-liver oil added to the diet. A southern exposure was used and the windows were not washed or cleaned during the progress of the experiments. Starting in January, 1928, all eggs from each of these groups were gathered and incubation of the weekly product made. Curves giving the relationship between the percentage production, percentage fertility and weekly period of incubation showed that the best production and fertility of eggs were obtained with stock housed behind ordinary window glass with 2% by weight of cod-liver oil added to the standard Wisconsin ration, followed closely by the results from stock housed behind quartz-containing glass (vitaglass or helioglass). The production and fertility of eggs from hens kept behind ordinary window glass and fed the standard ration without cod-liver oil fell off gradually from January to April, finally decreasing to 40 to 50% of the values obtained with hens kept under the other 2 conditions. These experiments indicate that: (1) there is evidently sufficient ultraviolet light transmitted by such quartz-containing glass as vitaglass or helioglass in the winter months in Minnesota to keep production and fertility of eggs at a high level; (2) 2% by weight of cod-liver oil is slightly superior in the results obtained as compared to the use of quartz-containing glass such as vitaglass or helioglass, and (3) ordinary window glass screens out irradiation from winter sunlight which is beneficial to the maintenance of high production and fertility of eggs.

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Potentiometric and Spectrophotometric Changes in Plants Produced by Infra-Red and Ultraviolet Irradiation.*

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Potentiometric changes. —Various types of electrodes, to be described later, were used in the work to be reported. In general, however, electrodes of thin tinfoil were used and were attached to the leaves or stems of plants by means of acid-free kaolin ground in a mortar with a little water and glycerine after the method of Sen.[1] In investigating potentiometric changes in leaves, both before and after irradiation, one electrode was fastened near the base of the leaf and the other one was placed at or near the tip of the leaf. Leads were then carried to a Leeds and Northrup potentiometer, using a high sensitivity galvanometer.

Various spectral filters, such as Corning Glass Company filters of special make (G 554 FF, G 24, G 34 R, G 584, G 586 A, G 586 AW, G 392, G 984 B, G 985 B, G 986 A) and transmitting only infrared, red, blue and ultraviolet rays, and so forth, respectively, were employed.

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* The literature bearing on electrical potentials developed in plants is not given. Reference, however, should be made to Scurti and Cortese, *Ann. Chem. Applicata*, 1927, xvii, 407-414; Taylor and Whitaker, *Protoplasma*, 1927, iii, 1-6, and Lund and Kenyon, *J. Exp. Zoöl.*, 1927, xlviii, 333-357. The researches of C. M. Child and W. J. V. Osterhout regarding the occurrence and nature of gradients are both numerous and valuable. An excellent résumé regarding bioelectric potentials and currents is to be found in *Protoplasma*, 1928, iii, 447.

[1] Sen, *Proc. Roy. Soc.*, 1922, xciv, 216.

Infra-red (heat) energy, as such, could be removed from the sources of illumination (such as the quartz-mercury arc) by water cells of sufficient depth and possessing quartz bottoms. The visible and infra-red portions could be removed by water cells and solutions of certain substances or by special mediums transmitting selected portions of the ultraviolet light only.

The sources of energy used were infra-red lamps and arcs rich in ultraviolet irradiation. A large number of determinations on the energy received by the leaf under irradiation (from various sources and with various filters) was made as well as determinations of the relative energy of various lines of the spectrum of the quartz-mercury arc when operated at various voltages. Such measurements must be obtained if any attempt is to be made to correlate biologic reactions and reception of energy. So far as we know, this is the first attempt to correlate quantity and quality of incident energy with electrical and other changes in plants.

Sunflowers were used in general for experimental purposes during the summer; poinsettias were found satisfactory during the autumn months.

Although the experimental results have not been wholly consistent, the data obtained during the summer and autumn of 1927 and 1928 definitely show that marked changes in electromotive forces in leaves are produced by irradiation with infra-red and ultraviolet rays. The potential changes under heat rays from an infra-red burner are rapid and values as high as 0.3 volt change in potential have been obtained. The potential changes produced by irradiation with a quartz-mercury lamp have been similar to and in the same direction as those produced with infra-red irradiation. The visible portions of the spectrum of air-cooled quartz-mercury arcs operated at 90 volts did not produce changes, except small changes in the region of the short visible waves (violet end of spectrum). Irradiation with quartz-mercury arcs from which the infra-red energy had been removed by suitable water filters, hence allowing the incidence of visible and ultraviolet rays only, produced potential changes in the same direction as those produced by infra-red rays and of an order ranging from 0.005 to 0.10 volts. These data show that potentiometric changes are produced by infra-red and ultraviolet irradiation. The full significance of these results and their possible explanation (that potentiometric changes induced in the cells in the surface layer only of the leaf are being measured) will be discussed in the complete papers.

In general, the relationship between the response, *R*, as measured by the change in potential difference produced by irradiation and the stimulus, *S*, of given spectral quality and quantity (that is, $S = kIt$, when *I* is the intensity of illumination and *t* the time of irradiation in minutes) is expressed by the equation $R = c \log S$. Equal quantities of energy in various portions of the spectrum as measured by radiometric methods (such as thermopiles), do not produce the same potentiometric changes in plants.

Ozone and oxides of nitrogen produced by the quartz-mercury arc or by other metallic arcs, operating at high voltages, through which air was drawn, the ionized or nascent gases then being conducted through suitable channels to the container surrounding the leaf under test, showed marked potentiometric changes and in the same direction as the changes produced by infra-red or ultraviolet irradiation. These observations lend support to the theory which we are advancing that ionization, due directly to ultraviolet light or indirectly to the ionized condition of the gas (air) which forms the medium surrounding the leaf, is responsible for the potentiometric changes observed. The rôle of loss of water under infra-red irradiation and the possible production and action of hydrogen peroxide under ultra-violet light in the presence of ionized gases will be discussed at length in the complete papers.

Spectrophotometric changes. The spectrophotometer built by Keuffel and Esser was used in the study of the color changes produced in leaves when exposed to radiation of various qualities and quantities. The type of water-cooled attachment, devised by Brunsting and Sheard, carrying the magnesium carbonate block which serves as a standard for reflection of light, was employed in this work because it enabled us to obtain spectrophotometric measurements from living leaves, intact with the plant, over as many periods and under as many conditions as desired.

After a few minutes' exposure of a given area (usually 2 by 4 cm.) on a leaf (intact plant) to infra-red irradiation it was found that considerable darkening of the exposed area occurred shortly after irradiation. The degree of this darkening increased with time until it finally reached a steady state. Repeated exposures of various leaves of different plants to irradiation with red, yellow, green and blue portions of the visible spectrum did not produce changes in spectrophotometric reflection. Ultraviolet rays, especially those below the limit of sunlight (practically speaking, 300 millimicrons), produced a definite bronze or brown-black effect in general. This was always true with the sunflower, but some exceptions, with actual apparent bleaching or equivalent loss of ordinary chlorophyll, occurred under certain conditions in the leaves of poinsettias. Hence plants, as well as human beings, get a browned and darkened epidermis by exposure to ultraviolet rays. Microscopic examinations showed that these effects were accompanied with a breaking down of the outer layers of cells of the affected areas. Numerous changes in character and degree of coloration (browning or blackening) can be produced, however, by control of such factors as character of irradiation, supply of water to plant, humidity of surrounding air, presence or absence of ozone, and temperature.

4427

Motility of the Empty Stomach in Normal and Malnourished Asthenic Children.

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The problem is concerned with the motility of the empty stomach in normal children and in those who are of the asthenic type and in a malnourished condition, being underweight for the age and height. This preliminary report includes studies on 5 normal and 14 asthenic children. The age range was 4 to 14 years. In addition, there was one 17-year-old girl whose development and reactions were those of a child of 12 years.

The method used was that of Carlson.[1] A deflated balloon attached to one end of a rubber catheter was swallowed. The other end was attached by means of hard rubber tubing to a chloroform manometer fitted with a lever arranged to write on a moving smoked paper. The balloon was inflated at the beginning of the experiment and the pressure changes due to waves of contraction in the stomach were recorded. The experiments were run in the morning before breakfast after the child had fasted 14 to 16 hours. At least 3 experiments were performed on each child, each one lasting from 1 to 2½ hours. Those in which vomiting and gagging occurred were discarded. Care was taken to have the child in good condition, determined by a restful preceding night, and a normal temperature.

The tracings as taken included records of the respirations and heart beats as well as the contraction waves of the stomach as described by Carlson.[1] Variations in the types of contractions as well as in the sequence of types I, II, III, of the thirty-second rhythm and of the twenty-second rhythm were obtained. In order to compare the activity of the normal and asthenic children a method of analysis of the tracings had to be worked out. This was done by

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[1] Carlson, A. J., "The Control of Hunger in Health and Disease," Chicago, 1916.

TABLE I.

Comparative Activity of the Empty Stomach in Normal and Malnourished Children

	No. Subj.	No. Exp.	Low activity (1 & 2 & 3) average	Medium activity (4 & 5) average	High activity (6 & 7) average	High tonus (8) total	Tetanus (9) total
Normal children	5	10	14.9 min	59.9 contractions in 41.3 minutes	20.9 contractions in 12.9 minutes	10 periods (24 contractions) in 12.5 minutes	6 periods (126 contractions) in 46 minutes
Asthenics without gastro-intestinal complaints	11	27	33.9 min.	33.5 contractions in 25.5 minutes	9.3 contractions in 7.3 minutes	2 periods (8 contractions) in 3 minutes	
Malnourished Asthenics with gastro-intestinal complaints	3	10	55.2 min.	29.9 contractions in 19.3 minutes	2.9 contractions in 1.9 minutes		1 period (14 contractions) in 4½ minutes

dividing a complete cycle of activity into 9 periods, ranging in order from lowest to highest activity as follows: (1) no waves, (2) tonus waves only, (3) twenty-second rhythm, (4) thirty-second rhythm, Type I, (5) Types I and II mixed, Type I predominating, (6) Type II and I mixed, Type II predominating, (7) Type II only, (8) high tonus with superimposed contractions, (9) tetanus. The number of contraction waves in each period was also recorded. For simplicity, these periods were arranged in groups of low, medium and high activity, in each being recorded the averages of the number of contractions and the duration in minutes. The group of low activity includes periods 1, 2, 3; medium-periods 4 and 5, high-periods 6 and 7. The total number of periods of high tonus (period 8) and tetanus (period 9) are given because the number is too small to be averaged.

The subjects were divided into 3 groups, (1) normal children, (2) malnourished asthenics without gastro-intestinal complaints, (3) malnourished asthenics with complaints such as anorexia, nausea, vomiting, abdominal distress, etc. X-ray examinations of the gastro-intestinal tracts of the children in group 3 were negative. The results are shown in Table I.

From the table it can be seen that the activity in the asthenic children is less than that in normals, judged by the duration of active periods and the number and type of contractions. The most striking difference is in the tetanus periods, this being the highest possible form of activity. In a total of 10 experiments on normal children, 6 tetanus periods with 126 superimposed contractions, total duration 46 minutes, were obtained, while in the 37 experiments on asthenics, only one short tetanus period lasting 4½ minutes, with 14 contractions, occurred.

Whether the lowered activity in the asthenics is peculiar to the type or is a part of the generally poor nutritional condition, or whether the low activity is responsible for the malnutrition, are questions still to be answered.

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The Action of Volatile Fatty Acids on the Gastric Motor Mechanism in Dogs.

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A fat splitting enzyme lipase has been demonstrated in the normal gastric secretion. Mathews[1] states that the digestion of emulsified stomach fats occurs to the extent of about 50 to 80% of the available total. Starling[2] showed that a considerable amount of fat is broken down in the stomach following a fatty meal. Evidence of fat hydrolysis was the presence in the stomach contents of a large proportion of free fatty acids. Edelstein and Czonka[3] found some volatile fatty acids present, including butyric, caproic, caprylic and acetic acids in infants' stomachs.

Bahrdt and Bamberg[4] found that small amounts of lower volatile fatty acids, given by mouth produced increased peristalsis in the stomach as seen by fluoroscopic examination. The effect on the small intestines as reported by Bokai[5] was to increase peristalsis. Catel and Graevenitz[6] found the action of butyric acid on intestinal motility variable. In low concentrations it was sometimes stimulating, at other times inhibitory. In higher concentrations it was always inhibitory. Babkin[7] reported that fatty acids were stimulating in some portions of the intestines and inhibitory in other parts.

Carlson[8] reported the effects on gastric motility of various acids introduced into the stomach of dogs and man, but did not use the volatile fatty acids.

This work was undertaken to determine the effects of butyric, caproic, caprylic and caprinic acids on the tonus and motility of the gastric motor mechanism in dogs.

The experiments were carried out on young, healthy, female dogs having a gastric fistula. Four different dogs were used and more than a hundred observations made. Changes in motility and tonus were recorded by means of an intragastric balloon connected to a manometer recording on a kymograph. Various concentrations of butyric, caproic, caprylic, and caprinic acids were used. Solutions of .01% to 5% concentrations were tested. 5 cc. of the acid dilutions were placed inside the stomach by means of a glass pipette introduced through the fistulae. Observations were made on both the quiescent and the active stomach.

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[1] Mathews, Albert P., *Physiol. Chem.*, 1916, 2nd ed., 350.

[2] Starling, M. D., *Guy's Hospital Reports*, 1910, 165.

[3] Edelstein, F., und v. Czonka, F., *Z. f. Kinderh.*, 1912, iii, 312.

[4] Bahrdt and Bamberg, Karl, *Z. f. Kinderh.*, 1912, iii, 322.

[5] Bokai, *Arch. f. Exp. Path. u. Pharmak.*, 1888, xxiv, 153.

[6] Catel, W., und Graevenitz, F., *Jahrb. f. Kinderh.*, 1925, cix, 249.

[7] Babkin, B. P., *Canad. Med. Assn. J.*, 1928, xviii, 267.

[8] Carlson, A. J., "Control of Hunger in Health and Disease," Chicago Univ. Press. 1916.

Following the introduction into the quiescent stomach of solutions of butyric acid, from .01% to 0.4%, no effect on the gastric tonus or tonus rhythm was noted. With concentrations of 0.5% there was an immediate fall in gastric tonus with little or no inhibition of the tonus rhythm. With higher concentrations of butyric acid, up to 5%, a constant fall in tonus with a variable inhibition of tonus rhythm occurred. Following the initial sharp fall in tonus and inhibition of tonus rhythm, there was a slow rise of tonus and a gradual return of rhythm to the level and amplitude present at the onset of the experiment. The fall in tonus was from 1 to 2 cm.; while the duration of inhibition was from 1 to 15 minutes.

In the fasting stomach, during periods of activity, the introduction of 5 cc. amounts of dilute butyric acid had no effect. With 0.5% concentration there was a quick fall in tonus with incomplete inhibition of the gastric hunger contractions. With higher concentrations of the acid, up to 5%, the fall in tonus was constant, while inhibition of hunger contractions became stronger until complete inhibition of the hunger waves occurred. The fall in tonus was from 1 to 4 cm., with recovery in 1 to 5 minutes.

The duration of the inhibitory action on the hunger contractions was from 2 minutes to an hour or more. Recovery of activity after total inhibition occurred in the following sequence: First, recovery of tonus to its primary level. Next return of tonus rhythm, then the return of hunger contractions of increasing amplitude. Caproic acid was less markedly active in inhibitory effect than butyric. In concentration of 4%, however, it usually produced complete inhibition. Caprylic acid was comparable to caproic acid in its effect. Caprinic acid was the least active of the group tested. In concentrations of from 3 to 5%, however, it was also inhibitory.

4429

A Natural Infection of Quail by B. Tularense.

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Studies indicating that several species of game birds are susceptible to experimental invasion by *B. tularense* have previously been reported by Parker and Spencer[1] and by Green and Wade.[2, 3] These findings indicated that a careful search might reveal natural cases of tularemia in birds.

Parker has recently demonstrated that experimental tularemia may be produced in quail by feeding infective material. The infections so produced were fatal. In a report of this work, Parker[4] cites 2 cases of human tularemia in which the infection may have been obtained from quail.

The experimental demonstration and subsequent isolation of *B. tularense* in a quail dying in the wild is here reported. A flock of 10 or 12 quail living near the outskirts of Minneapolis was under the observation of near-by residents. On February 17th, at the height of Minnesota winter, the covey, which had apparently decreased in number to 6, was accidentally flushed. One bird was unable to fly and was picked up. A second flew blindly into the side of a building but resumed flight in another direction. The 4 remaining birds were described as having an unsteady and unnatural flight. The captured quail was taken inside a house and died during the night. The next day it was brought to the laboratory for examination. As the carcass was frozen, necropsy was delayed until the following day. This quail is designated as No. 1.

On February 21st, another resident of the community followed the tracks of a single quail and found the bird lying dead at the bottom of a snow bank. He buried the carcass, which was already frozen, in the snow bank. Three days later it was picked up, but was not brought to the laboratory for necropsy until February 26th,

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[1] Parker, R. R., and Spencer, R. R., *Sixth Biennial Report of the Montana State Board of Entomology*, 1925-1926, 30.

[2] Green, R. G., and Wade, E. M., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 515.

[3] Green, R. G., Wade, E. M., and Kelly, W., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 260.

[4] Parker, R. R., *Public Health Reports*, in press.

5 days after the bird was found dead. This quail is designated as No. 2.

Quail 1. At necropsy this quail was found very emaciated. Careful examination failed to show macroscopic lesions in any of the organs. A single whip worm was found in the intestine. A guinea pig was injected with emulsified liver subcutaneously in the left inguinal region. A pigeon was inoculated intramuscularly with the same emulsion. The guinea pig died on the sixteenth day after inoculation, with the left inguinal nodes greatly enlarged and infiltration in the subcutaneous tissues of the left inguinal region. The right inguinal region was normal. The liver and spleen were thickly studded with nodules typical of tularemia. Pieces of liver and pieces of spleen from this guinea pig were emulsified and inoculated into 2 guinea pigs each, all 4 guinea pigs dying on the fourth day following, with lesions typical of tularemia. From one of the guinea pigs inoculated with liver, *B. tularense* was isolated in pure culture. The characteristics of this culture appeared typical, and it was further identified by specific serum agglutination. The pigeon inoculated with liver from quail No. 1 did not develop any symptoms. Its blood contained no agglutinins 18 days after inoculation, and at necropsy, 19 days after inoculation, it appeared to be normal.

Quail 2. Upon necropsy quail No. 2 appeared to be in a state of good nutrition. Careful examination failed to show any macroscopic lesions or the presence of parasites. An emulsion of liver injected into a guinea pig failed to produce glandular enlargement or symptoms of illness, and upon necropsy, three weeks later, the guinea pig appeared to be normal.

Of the 6 quail observed after disease was known to be present in the covey, 2 only are known to have died, but none of the others have since been seen. The fact that the pigeon did not develop any disease upon inoculation is evidence that no infectious disease other than tularemia was present in quail No. 1. The failure to isolate *B. tularense* from quail No. 2 cannot be considered a conclusively negative finding in view of the lapse of time between death and necropsy. The presence of *B. tularense* in one bird of a covey which apparently was afflicted with disease and was known to be diminishing in size, would indicate tularemia as the probable destructive factor.

Western New York Branch.

University of Buffalo School of Medicine, April 17, 1929.

4430

Effect of Adrenalin and Pituitrin on the Volume of the Liver.

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In view of the contradiction which exists in the literature in regard to the effects of adrenalin and pituitrin on the circulation in the liver, we have used the liver plethysmograph, which has been previously described,[1] in a new attack on this problem. The results will be very briefly described in this preliminary report without substantiation from actual records or attempt to cite the literature; these will appear in due time.

Cats and rabbits under chloralose anesthesia have been used in this work; the results are as follows:

I. Intravenous injections of adrenalin. 1. Pressor doses invariably produce constriction in the liver, whether introduced by way of the femoral, or portal veins, or hepatic artery, provided the general blood pressure is high and the animal in good condition. This constriction may last as long as the pressor response or it may at times give way to prompt and active dilation; when this latter occurs it may be abolished by section of the depressor nerves (rabbit). On the other hand, if the animal is in poor condition with low blood pressure the liver apparently dilates passively during the pressor response to an injection by way of the femoral vein. This is intensified by first clamping the hepatic artery, but is prevented altogether and even replaced by constriction if the portal vein has been previously clamped so as to restrict the volume changes in the liver to variations in its arterial blood supply; *i. e.,* the liver is forced to expand under these conditions and in spite of active vasoconstriction within itself, because of an engorgement through its massive portal supply. This in turn is probably due to an unusual movement of blood through the toneless(?) mesenteric capillaries as a result of the high general blood pressure.

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[1] Griffith, York and Zachmys, Proc. Soc. Exp. Biol. and Med., 1928, xxv, 399.

2. When introduced by way of the hepatic artery or portal vein the dosage may be so adjusted as to produce vigorous constriction within the liver without having any effect on the general blood pressure.

3. Finally, very small doses by way of the femoral or portal veins may produce dilation of the liver with or without a fall in the general blood pressure.

II. Pituitrin, one-fifth cc. or more (Parke-Davis, obstetrical) produces about the same decrease in liver volume whether introduced by way of the femoral or portal veins or hepatic artery.

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Urobilinogen Concentration in Urine Specimens Containing Bilirubin.

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Gmelin's test for bile pigments was carried out on all specimens of urine submitted to the laboratory during a period of a little more than 2 years. Approximately 50,000 specimens were examined during this time, and a positive test was obtained in 476 specimens from 308 subjects. Most of the patients treated in this institution are suffering from chronic diseases and are of fairly advanced age. Since daily specimens were obtained from diabetic patients and urine from those on the surgical service was examined routinely 3 times a week these 2 groups have an undue prominence in the series. The approximate concentration of urobilogen in the specimens giving a positive Gmelin test was determined by the method of Wallace and Diamond.[1] Normal urines show a positive test by this method when diluted 1 to 10 or 1 to 20 with water, but give no color when dilution is greater. In our series 142 specimens, or less than a third of the total, fell into this range. There were 54 specimens from 18 patients which gave a negative Wallace and Diamond reaction, and it seemed probable that these could be adequately explained by failure of bile to enter the intestine, as the diagnoses indicated the certainty or the possibility of the presence of lesions obstructing the bile ducts in most cases. Only 24 specimens from 22 patients showed a positive reaction which became negative when the urine was diluted with 9 parts of water. Over half of the analyses (256 specimens) gave results which were high, and such figures were obtained at some time or other upon 198 patients, or approximately two-thirds of the entire series. In general the diagnoses on these patients offered no adequate explanation of the findings. Too fine an interpretation should not be placed upon such results based upon clinical pathological tests, but certain tentative conclusions can nevertheless be drawn from the figures. At the beginning of our study is seemed probable to us that most increases in the bilirubin content of such urines as we were examining arose from a decrease in the efficiency of the excretory power of the liver. Such a change might be either primary or secondary to the general circulatory disturbances which are not infrequently seen among older patients who are suffering from chronic diseases. The results suggest that bile is entering the intestinal canal in fairly large amounts, so this explanation is perhaps incorrect. If, however, the liver ordinarily removes both pigments from the blood stream,[2] increases in both should be expected not infrequently. This idea seems to explain satisfactorily the simultaneous excretion in the urine of increased amounts of both urobilin and bilirubin.

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[1] Wallace and Diamond, *Arch. Int. Med.*, 1925, xxxv, 698.

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[2] Wilbur and Addis, *Arch. Int. Med.*, 1914, xiii, 235.

Pacific Coast Branch.

Hopkins Marine Station, Pacific Grove, April 20, 1929.

4432

Effect of Insulin on Protein Metabolism. The Rôle of Hypoglycemia.*

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In our studies of the effect of insulin upon protein metabolism we have been concerned most recently with the rôle of hypoglycemia in relation to the changes in the amino acid content of the animal. It was demonstrated by Kiech and Luck[1] that insulin decreased the amino acid nitrogen and increased the rate of urea formation but the extent to which these effects were secondary to the accompanying hypoglycemia was not determined. The experiments which we have conducted are similar in principle to those of Kiech and Luck. Standard albino rats in groups of 6 were used. Each group was divided at the time of experiment into 2 sets of 3 each. The members of the first set received insulin subcutaneously (6 units per kilo). Immediately after, 1 cc. of 20% glucose was administered A second injection of the same amount of glucose was made an hour later. The control animals received insulin only, 1 cc. portions of 0.9% sodium chloride being given in place of the glucose administered to the experimental animals. One half hour after the last injection the bladders were emptied and the animals placed in individual, small, metabolism cages. Thereafter the animals constituting a set, were killed at intervals of 1, 3, and 4 hours. The whole carcass, including any urine voided within the time, was analyzed for amino acid nitrogen and urea. Forty-nine animals were used. The results are presented in Table I.

As was observed by Kiech and Luck, insulin alone was found to decrease the amino nitrogen content of the animal. The administration of glucose with the insulin did not nullify the characteristic

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* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[1] Kiech, V. C., and Luck, J. M., *J. Biol. Chem.*, 1928, lxxviii, 257.

TABLE I.
The Effect of Insulin on the Amino Nitrogen Content of Rats.

Insulin	Saline	Insulin and glucose	Insulin
36.8	44.8	39.5	36.5
40.2	50.1	39.9	38.8
43.9	43.9	37.4	41.6
39.5	49.5	45.0	36.5
41.8	47.4	36.3	39.7
40.2	46.4	36.5	43.0
41.9			
		38.0	39.4
42.0	47.1	37.4	37.9
42.5	46.5	38.2	38.2
42.4	47.0		
		47.0	36.5
35.4	40.5	36.5	44.2
32.2	37.9	40.2	41.9

insulin effect. Although these experiments are preliminary in nature, they necessitate the conclusion that the effects of insulin on protein metabolism are not secondary to the accompanying hypoglycemia but are probably primary phenomena. The final report will include the results of urea estimations and experiments in which the hypoglycemic action of insulin has been combated in other ways.

4433

Infection of Monkeys With Poliomyelitis Virus by the Gastro-Intestinal Route.

E. W. SCHULTZ.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

Aycock[1, 2] has recently reported 3 outbreaks of poliomyelitis which he believes can be traced to contaminated milk. The observations recorded by Aycock are of particular interest in the face of essentially negative results which have been realized under experimental conditions. Leiner and v. Wiesner[3] succeeded in producing the disease in only 2 out of 5 monkeys fed with large quantities of the virus, after neutralizing the hydrochloric acid of the stomach with sodium bicarbonate and reducing the motility of the intestines

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[1] Knapp, A. C., Godfrey, E. S., Jr., and Aycock, W. L., *J. Am. Med. Assn.*, 1926, lxxxvii, 635.

[2] Aycock, W. L., *Am. J. Hyg.*, 1927, vii, 791.

[3] Leiner, C., and v. Wiesner, R., *Wien. Klin. Woch.*, 1910, xxiii, 91.

with morphia. Following the same procedure, Amoss[4] obtained entirely negative results, even though the monkeys were fed a considerable quantity of the virus over a period of 42 days. The animals not only failed to develop the disease, but also failed to acquire an immunity as a result of the massive ingestion of virus material. Levaditi and Landsteiner[5] were unsuccessful in producing infections in monkeys fed with virus-contaminated milk. No immunity was noted on subsequent intracerebral inoculation.

Though the results reported appeared to indicate quite clearly that in monkeys, at least, infection by the gastro-intestinal tract is extremely difficult, if not practically impossible, nevertheless, the important observations made by Aycock seemed to justify a repetition of feeding experiments in monkeys.

We have accordingly endeavored to infect 2 normal monkeys with heavily contaminated milk. In order that natural conditions might be maintained, nothing was previously done to the monkeys except to subject them to a brief period of fasting. The animals then had placed before them approximately 150 cc. of milk containing about 7 gm. of a finely ground cord and medulla of a monkey having recently succumbed to the disease (P.M. No. 44). This the animals consumed entirely within 2 hours. Several hours later (in the evening) a similar quantity of virus in milk was placed before them, this time prepared from the cord and medulla of another monkey (P.M. No. 70) having succumbed to the disease. This was consumed by the following morning. At noon they received another dish of milk containing the same quantity of virus material (P.M. No. 68). The feeding was discontinued at this point. The animals were kept under observation from November 23rd until February 2nd, during which time they remained perfectly normal. On February 2nd each received an injection of 0.75 cc. of a 5% virus-cord suspension intracerebrally. One of the monkeys (P.M. No. 48) developed typical poliomyelitis on the fifth day, the other on the seventh day. These monkeys were, therefore, still fully susceptible to the usual mode of inoculation. The strain of poliomyelitis employed in these experiments is a fully virulent, and highly dependable strain originally isolated by Aycock, and supplied to us through the courtesy of Dr. Flexner.

These observations tend to show that it is quite impossible to infect monkeys by the gastro-intestinal route, even though a poliomyelitis virus is employed which regularly produces an infection in monkeys when administered by the intracerebral route. They, however, do not necessarily invalidate the possibility that the disease in man may at times be spread through the medium of milk. One must keep in mind that poliomyelitis is not a natural disease of monkeys. From our experience and those of others, it is apparently impossible to infect fully susceptible normal monkeys by caging them with incubational and sick animals. Monkeys must, therefore, enjoy a relative immunity to the disease, an immunity which is broken down only by such artificial, and relatively brutal, methods as intracerebral inoculation. The fact that monkeys are not naturally susceptible to the disease makes the solution of some of the more important questions as they relate to the human disease extremely difficult. Among such problems, for example, is an effective method of immunization against the naturally acquired disease. Whatever the intrinsic merits of any procedure may be, in so far as it applies to the protection of man against natural contact infection, it is perforce obscured in the laboratory experiment by the relatively drastic procedures necessary to induce the disease in monkeys. This condition undoubtedly also influences the solution of such epidemiologic questions as the one just referred to. The fact that man is naturally susceptible to the disease, and that monkeys are not, must therefore unquestionably occupy a prominent place in any consideration which has to do with the application of observations made on monkeys to the circumstances as they apply to man.

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[4] Amoss, H. L., Filterable Viruses, Rivers *et al.*, Williams and Wilkins, Balt., 1928, 174.

[5] Levaditi, C., *Ectodermoses Neurotropes. Poliomyélite, Encéphalite, Herpès.* Monograph l'Institut Pasteur, Masson et Cie, 199, 66.

4434

Relationship of Atopens of Timothy (Phleum Pratense) and Australian Rye (Lolium Multiflorum) Grasses as Indicated by Passive Transfer Studies.

EDWARD MATZGER. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco.

There is an assumption that the atopens of timothy grass pollen contain atopens that will protect patients who are sensitive to other grasses. The basis for this belief has been the clinical evidences of satisfactory results. Coca and Grove added the weight of experimental evidence to this hypothesis when they demonstrated that the timothy atopens contained the atopens of June, orchard and red top grasses. Botanical surveys of the San Francisco and peninsula areas indicate that Australian rye grass pollen is an important factor in pollen hypersensitiveness. It was of interest to determine whether the timothy atopens might contain the rye grass atopens and this question was studied experimentally on human beings by means of the local transfer technic. The observations which will be detailed elsewhere show definitely that the timothy atopen does not contain the rye grass atopen while, on the other hand, the rye grass atopen does contain the timothy atopen. It is quite evident that the treatment of patients with timothy pollen extract alone will not protect patients who are clinically exposed to rye grass pollen. On the contrary, however, a definite group of patients who are exposed to timothy pollen may be protected by the use of rye grass extract.

Missouri Branch.

St. Louis University School of Medicine, April 24, 1929.

4435

Effect of Potassium Iodide and Desiccated Thyroid Substance on Metabolic Rate of Guinea Pigs.

J. CORDONNIER. (Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

A study was made of the changes in the basal metabolism of guinea pigs under the influence of the administration of potassium iodide, thyroid substance, and of a combination of potassium iodide and thyroid. All determinations were made with an apparatus devised by Foster and Sundstroem,[1] and the results are expressed as calories/kilo guinea pig/hr.

Our purpose was to correlate the metabolic changes, or their absence, with previous microscopic studies made in our laboratory on the thyroid gland of guinea pigs under the influence of iodine, and of the other substances mentioned.

Loeb[2] has found that oral administration of thyroid substance to guinea pigs in which a considerable part of the thyroid gland had been removed, prevents compensatory hypertrophy from taking place, depressed activities in the gland, and mitotic activity is diminished. On the other hand, administration of KI does not prevent hypertrophy, it favors the solution of the colloid, and the hypertrophy is, on the whole, apparently greater in the gland of those animals which have received this drug. In a similar way, Gray and Loeb[3] found KI to increase very markedly the proliferation of the gland and also slightly to increase the size of the epithelium and to soften somewhat the colloid.

In view of these structural changes, it was of interest to determine whether there was a corresponding alteration in the basal metabolic rate. It should be expected that alteration of so pronounced a character in a gland which largely controls the metabolic rate of the organism would affect that rate.

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[1] Foster, G. L., and Sundstroem, E. S., *J. Biol. Chem.*, 1927, lxix, 565.

[2] Loeb, Leo, *J. Med. Res.*, 1920, xli, 481.

[3] Gray, S. H., and Loeb, Leo, *Am. J. Path.*, 1928, iv, 257.

Thirty-six guinea pigs, weighing between 350 and 500 gm., were used. During the experiment which in the majority of cases extended over a period of thirty days, repeated metabolic studies on each animal were made and a careful record of changes in weight was kept.

Kunde,[4] Snell, Ford and Rowntree,[5] and Liebesney[6] have studied metabolic changes following KI administration, both in human cases and in animals. All conclude that in normal individuals the change may be quite variable, and that it is never very great. Marine, Deutch, and Cipra,[7] experimenting with 18 normal rabbits, found a drop in metabolic rate in 5, a slight increase in rate in 2, and no appreciable change in the remaining 11. Webster and Chesney[8] found a decrease in metabolic rate after the first dose of Lugol's which attained a maximum on the third day, after which there was a tendency to return to a normal level.

In 10 animals, over a 30 day period, we found that oral administration of KI had no appreciable effect on the basal metabolic rate, but that there may be some individual variation both above and below the normal averages. The weights of the animals remained comparatively unchanged.

As was to be expected, there was a definitely increased metabolic rate, with an accompanying loss of weight, in the 11 animals which received thyroid substance alone. The maximum metabolic level was practically attained during the first 10 days and there was only a slight increase in metabolism between the tenth and twentieth day. These changes are clearly demonstrated in the averages shown on the accompanying table.

In addition to the guinea pigs which received KI alone, this substance was also administered to animals which had previously received thyroid substance over a 20 day period. A similar number of animals were given no KI when the thyroid feeding was discontinued and were used for controls. The aim of this experiment was

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[4] Kunde, M. M., *Am. J. Physiol.*, 1927, lxxxii, 195.

[5] Snell, A. M., Ford, F., and Rowntree, L. G., *J. Am. Med. Assn.*, 1920, lxxv, 515.

[6] Liebesny, P., *Wien. klin. Wchnschr.*, 1924, xxxvii, 521.

[7] Marine, D., Deutsch, M., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 657.

[8] Webster, B., and Chesney, A. M., *Bull. Johns Hopkins Hospital*, 1928, xliii, 291.

TABLE.

	Begin		1-10 days		10-20 days		20-30 days	
	Weight	B.M.R	Weight	B.M.R.	Weight	B.M.R.	Weight	B.M.R.
Controls	383	4.11	370	4.45	374	4.35	377	4.14
Administration of KI	358	4.58	349	4.77	369	4.47	419	4.32
Administration of desiccated thyroid	402	4.24	350	6.03	346	6.26		
Administration of KI and thyroid	397	4.05	341	5.82	344	6.04	377	5.99

Animals to which KI was not given after the thyroid feeding was discontinued.

Days	1	3	5
Average B.M.R.	5.86	5.24	4.33
Average decrease		.62	1.53

Animals to which KI was given after thyroid feeding was discontinued.

Days	1	3	5
Average B.M.R.	5.76	4.90	4.26
Average decrease		.86	1.50

* All B.M.R. results are expressed as Cal./kilo guinea pig/hr.

to determine whether KI influences the return of the metabolism to the normal level after cessation of thyroid feeding. In one case, the KI seemed to hasten the return to a normal level, but generally, we found that it exerted no influence on the return to a normal metabolic rate previously raised by the feeding of thyroid substance. All animals had attained a normal level in 5 days.

In a series of 9 animals, to which thyroid substance and KI were fed in combination during the same period, we found that KI has no effect on the rise in basal metabolic rate in experimentally induced hyperthyroidism. The rate in these animals was approximately the same as that of those animals to whom thyroid alone was fed.

Determinations were also made on a series of 5 controls. Here the results were constant and there was very little variation from the normal average in individual cases.

From our results, we may conclude that KI, notwithstanding its stimulating effect on the gland substance, does not lead to an increased output of thyroxin, for when KI is given either alone, or in combination with thyroid substance, it produces no noticeable alteration in the metabolic rate. Administration of thyroid substance, as is well known, increases the metabolic rate, especially during the first 10 days of administration of the substance due to a direct action, and not through its action on the thyroid gland.

To explain the results, we may, as Loeb[9] points out in a recent paper, consider the following possibilities: (1) KI stimulates the thyroid gland at certain periods of its action. At the same time, it prevents an excess of thyroxin thus produced from leaving the acini; instead it is at least partly retained in the gland. (2) The stimulation of the acini does not necessarily lead to an excess production of thyroxin. (3) Peripheral effects counteract the stimulating effect of the thyroid hormone.

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[9] Loeb, Leo, read before the Association for the study of Goiter, Dayton, Ohio, March 25, 1929. To be published later.

Of these assumptions, the first seems to be the most probable. However, further experimental studies must be made before we can accept this interpretation as definite.

4436

Effect of Irradiated Ergosterol on Mammalian Thrombocyte Counts.*

R. A. PHILLIPS and D. F. ROBERTSON. (Introduced by E. A. Graham.)

From the Department of Surgery, Washington University School of Medicine and Barnes Hospital.

The suggestion for this work was found in the observations of F. D. Gunn,[1] who found that by exposing young rabbits to radiations from the mercury vapor lamp, a distinct increase in the number of thrombocytes was obtained in one week. Brougher[2] had demonstrated that large doses of cod liver oil administered to dogs with an experimental obstructive jaundice caused a return to normal of the coagulation time, which had been prolonged. Moise and Sooy[3] showed a rise in the thrombocyte count in thrombocytopenic purpura in human beings following irradiation with ultra violet rays over a period of 3 weeks.

The question arose in our minds as to whether or not irradiated ergosterol would produce a similar rise in thrombocytes. We did not concern ourselves with the coagulation time at the beginning of these experiments.

We chose rats, since Laurens and Sooy[4] had described a simple method for thrombocyte counts in these animals and charted what they found to be the normal counts. Five rats, male and female, of unknown age, were selected at random, and the erythrocytes and

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* We wish to acknowledge our indebtedness to the Department of Medicine and particularly to Dr. Thompson, whose timely advice and assistance has been invaluable.

[1] Gunn, F. D., Proc. Soc. Exp. Biol. and Med., 1926, xxiv, 120.

[2] Brougher, J. C., *Science*, 1928, lxviii, 256.

[3] Sooy, J. W., and Moise, T. S., *J. Am. Med. Assn.*, 1926, lxxxvii, 94.

[4] Sooy, J. W., and Laurens, H., Proc. Soc. Exp. Biol. and Med., 1924, xxii, 116.

thrombocytes were counted for several successive days until we obtained what we considered normal variations. The indirect method we used is as follows: The tail of the rat is carefully cleansed with soap and water and coated with vaseline. A small piece is snipped from the tip with vaseline coated scissors. The cut end is immediately immersed in the diluting fluid and a suspension of sufficient concentration obtained. The suspension of thrombocytes and red cells is then carefully shaken for 2 minutes and the counting chamber loaded therewith. The number of thrombocytes per cc. is determined from the ratio of reds to thrombocytes and the actual red count. Each thrombocyte count was done in duplicate, thus checking one against the other.

The preparation of irradiated ergosterol used was furnished to us through the courtesy of Mead, Johnson & Company, called "Acterol." They state that one drop is equivalent to 6 cc. of cod liver oil, but the actual amount of irradiated ergosterol per unit volume is not given. This material was given in drop doses by mouth.

The thrombocyte counts in rats receiving Acterol showed an immediate rise. With a dosage of 3 drops the thrombocyte count was double in 48 hours. Rats receiving 10 drops per day showed a similar rise in 48 hours, but the rise was not so high and with continued dosage dropped again after 2 days.

Our series of animals is very small and we do not, therefore, draw any sweeping conclusions. However, Dr. L. D. Thompson has kindly consented to let us report some of the results that he and his assistants have obtained using rabbits. They have been using a solution of irradiated ergosterol in olive oil, the content of the former being known, and have obtained similar results. We have been checking our first series of rats with a second and to date the results are the same. Consequently, we believe that our point has been proven, namely, that irradiated ergosterol increases the thrombocyte count in normal animals.

It is to be anticipated that the coagulation time, the bleeding time, and perhaps even the healing of wounds will be coincidentally affected and work on this point will be reported later. Some of the clinical aspects, particularly the reaction in purpura and other hemorrhagic conditions, are now being studied by Dr. Barr and Dr. Thompson of the Department of Medicine.

4437

Lactic Acid Increase in Muscle Under the Influence of Anesthetics.

HELEN TREDWAY GRAHAM. (Introduced by H. S. Gasser.)

From the Department of Pharmacology, Washington University School of Medicine, St. Louis, Mo.

The increase of lactic acid in resting muscle under the influence of narcotics is a familiar phenomenon with high concentrations of chloroform, and was observed by Meyerhof[1] for alcohol and urethanes in concentrations about 10 times those sufficient for general anesthesia. It has now been found that the lactic acid content of isolated whole muscle of green frogs is increased on the average 20 to 50% by ether, nitrous oxide and ethylene at tensions sufficient for general mammalian anesthesia (0.028 atmospheres ether vapor, 0.85 atmospheres ethylene, 1 atmosphere nitrous oxide). This increase is comparable to that produced by chloroform at an equivalent tension (0.01 atmospheres). The lactic acid increase is greater with higher tensions of these anesthetics, being 75 to 105% when 5 to 8 times the anesthetic tension is used. These increases occur with ½–2 hours exposure to the anesthetic, and do not seem to be greater with the longer exposures. They are measured by comparing the total lactic acid content of several small muscles (one of each of several pairs) exposed to narcotics, with the lactic acid content of the mates to these muscles not so exposed but otherwise similarly treated.

The possibility that the increased lactic acid content is caused wholly by narcotic interference with the oxidative removal of lactic acid is eliminated by the fact that the increase under the influence of narcotics, especially in high concentrations, is very much greater than the increase when all oxidations are prevented by cyanide; and by the second fact that treating both sets of muscles with cyanide before application of narcotic to one of them, or keeping them in an atmosphere of nitrogen throughout the experiment, seems not to decrease the difference between the amounts of lactic acid in the two sets.

These results suggest that the lactic acidemia of general anesthesia may not be due entirely to narcotic interference with oxidations, as is generally supposed, but may be due at least in part to the direct lactic-acid-producing action of the anesthetic on the muscle. The fact that the rate of fall of blood pH and of increase of blood lactic acid is maximal during the first part of a prolonged anesthesia may possibly be correlated with the experimental finding that the amount of lactic acid produced in isolated muscles in contact with narcotics does not seem to be much greater in 2 hours than in half an hour.

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[1] Meyerhof, *Pfl. Arch. f. Physiol.*, 1921, clxxxviii, 150; *ibid.*, 1921, cxci, 138.

The explanation of the increase of muscle lactic acid under the influence of narcotics seems to involve the structure of the muscle (it is not observed in muscle extract according to Meyerhof[2]) and may be formulated in terms of displacement by the narcotic of adsorbed lactic acid precursor or enzyme, with resultant freer contact between the 2 participants in the lactic-acid-producing reaction; or in terms of narcotic increase of membrane permeability, with the same result.

4438

Studies of Experimental Arthritis. III. Behavior of Skin Tests.

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The purpose of this study was to observe the behavior of skin tests under the conditions of experimental arthritis. One of the questions which a clinical study of chronic rheumatism presents is whether a joint once infected can in itself be a focus from which further exacerbations of the disease may arise. Accordingly, in arranging the experimental conditions under which the subject of bacterial allergy was to be studied, arthritis was produced not only by inoculating animals intravenously but also by inoculating small amounts of bacteria into the joints. The organism used was a laboratory strain of hemolytic streptococcus, preserved by freezing and desiccating the culture. Skin tests were made on the backs of the rabbits, after the hair had been clipped and removed with sodium sulfide. This depilatation was carried out 2 to 3 days before the skin tests were made. The toxic filtrate used was prepared by seeding a 24-hour broth culture of the organisms into about 100 cc. of Harley's toxin media. This was incubated for 5 days, centrifugalized and filtered through a Berkefeld candle. Normal rabbits were found to give no skin reaction following the intradermal injection of 1/10 cc. of these filtrates. Animals so tested were used in these experiments. In the series reported 24 animals were used, the same number being inoculated intravenously as by the intra-articular route.

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[2] Meyerhof, *Biochem. Z.*, 1926, clxxviii, 489.

After intravenous injection, rabbits usually develop a positive blood culture. Daily blood cultures then show one of two things. The animal tends to recover and the daily blood culture shows fewer and fewer organisms, or the animal grows rapidly worse with more and more organisms per cc. of blood and soon dies. The rabbits surviving may or may not develop metastatic joint infections.

Rabbits inoculated into joints rarely develop a positive blood culture; only one animal in whole series did, and it died on the fourth day. Blood cultures, however, in rabbits inoculated by the intravenous route and which have recovered, as well as animals inoculated directly into a joint, usually become positive just before death of the animal when death is due to some intercurrent disease.

All animals, in both routes of inoculation become allergic after the fifth or sixth day. This allergy or sensitization can be roughly measured by the size of the reaction and is represented in this paper by numerals the value of which were arrived at by taking the measurement of the discolored area in cm. in 2 diameters and using the product of the numbers.

In animals which were given organisms by the intravenous route, the allergy tends to grow less and finally disappear unless the animal develops some metastatic focus. In the animals with infected joints, allergy was present as long as the infection persisted. Spontaneous recovery from a metastatic joint infection was accompanied by a diminution in the allergic state as manifested by the skin reaction. Surgical draining of a joint, whether infected by the metastatic route or by direct inoculation was accompanied by the same result.

A dissemination of organisms by the blood stream is not necessary for the development of the allergic state as rabbits inoculated directly into a joint become allergic without ever developing a positive blood culture.

The pathological changes found both at the site of the focus and through the animal body were studied and reported in previous articles. However, autopsies were done on half of the present series and sections of lung, heart muscle, aorta, skeletal muscle, liver, spleen, and kidney examined. The pathology was found to be the same in rabbits inoculated by both routes and the same in allergic and non-allergic animals.

This pathological change consisted of a proliferative perivascular infiltration which did not show multinuclear cells.

4439

On the Particulate Size of Bacteriophage.

D. M. HETLER AND J. BRONFENBRENNER.

From the Department of Bacteriology, Washington University Medical School.

All existing evidence seems to indicate that the active agent responsible for transmissible lysis of bacteria is distributed in the medium as if it were particulate.[1] Several authors are inclined to consider these particles as autonomous units of an organized protoplasm.

In this connection it is of interest to measure and in other ways to identify these particles as such. Several attempts have been made to measure them. In most instances, these particles were[2, 3, 4, 5] forced through membranes of presumably known porosity. Because of the limited value of this method, von Angerer[6] tried to arrive at more reliable figures by the nephelometric method, and Bechold[7] used an ingenious gold cast method. While the figures obtained by these various procedures are not identical, they are sufficiently close to indicate, at least, that in all cases authors were dealing with particles of approximately the same order of magnitude.

A recent paper of Northrop and Anson[8] offers means to inquire into this question by the use of a different technique. By a suitable modification of the classical procedure for establishing diffusion rates,[9] they have proposed a comparatively simple method for obtaining the diffusion constants of complex biological substances. The size of the particles in solution can be calculated from these diffusion constants by the use of Einstein's equation.[10]

Following the procedure as used by them* for carbon monoxide hemoglobin, we have obtained the following results for *B. coli* bacteriophage:

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[1] Bronfenbrenner, J., *J. Exp. Med.*, 1927, xlv, 873.

[2] Prausnitz, C., *Klin. Woch.*, 1922, i, 1639.

[3] Stassano, H., and deBeaufort, A. C., *Compt. rend. Soc. de Biol.*, 1925, xciii, 1378.

[4] Eliava, G., and Saurez, E., *Compt. Rend. Soc. de Biol.*, 1927, xciv, 462.

[5] Zinsser, H., and Jang, F., *J. Exp. Med.*, 1927, xlvi, 357.

[6] von Angerer, *Arch. fur Hyg.*, 1924, xcii, 312.

[7] Bechold, H., and Villa, L., *Z. Hyg. u. Unfekt.*, 1925, cv, 601.

[8] Northrop, J. H., and Anson, M. L., *J. Gen. Phys.*, 1929, xii, 543.

[9] Oholm, L. W., *Z. physik. Chem.*, 1910, lxx, 378.

[10] Einstein, A., *Z. Electrochem.*, 1908, xiv, 235.

* We wish to acknowledge our indebtedness to Dr. Northrop for placing this method at our disposal in advance of its publication.

TABLE I.

Diffusion cell	Cell constant (K) as determined with 0.1 N HCl	Time in days	Phage diffused as cc. of original filtrate	D. for phage in cm.^{2}/days
No. 1	.1975	.0208	.00188	.0178
” 1	.1975	.0208	.00188	.0178
” 2	.1975	.0208	.000406	.0192
” 2	.1975	.0208	.000406	.0192
” 3	.1975	.0208	.00133	.0182
” 3	.1975	.0208	.0015	.0205
		Average		.0188

Using this average value of $D = (.0188)$ in the Einstein equation,[10] the radius of an average size particle of phage is calculated as having approximately the value of 6.1×10^{-7} cm. (or 6 $\mu\mu$). Of course, one has still to determine whether such particles represent the units of bacteriophage or serve merely as carriers for a more highly dispersed agent.[11]

4440

Differentiation Between Types of Fibers in Certain Components of Involuntary Nervous System.

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From the Departments of Surgery and Physiology, Washington University School of Medicine.

Criteria discussed in a previous report[1] have been employed to distinguish between 3 different types of fibers which occur together in nerves of the involuntary nervous system. Comparative measurements of threshold[2] have also been extended to these fibers. The cervical sympathetic trunk of one variety of turtle serves as a typical nerve containing such axons, recognizable by 3 groups of waves in their conducted action potential record on the oscillograph. These groups will be designated as the A, B, and C groups.

A second type of turtle has only 2 groups of waves in the cervical sympathetic nerve trunk, the second and third. Sections of the first type of nerve* show scattered, larger, and for the most part thinly myelinated fibers, with islands well marked off by trabeculae containing unmyelinated and small myelinated axons. Sections of the second type show only such islands containing practically no large fibers. The second and third groups of waves in these nerves are therefore inferred to correspond to the thinly myelinated and unmyelinated axons respectively of these islands. The relative areas of potential of the third wave in various other nerves studied corresponds in general to the relative number of unmyelinated axons. In the second type turtle the third wave group is larger than the second in area.

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[11] Bronfenbrenner, J., see pages 527-529 in the “Newer Knowledge of Bacteriology and Immunology,” Jordan and Falk, University of Chicago Press, 1928.

[1] Heinbecker, P., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 349.

[2] Bishop, George H., and Heinbecker, P., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 241.

* We are indebted to Mr. William Masek of the Department of Cytology for making sections of these nerves.

The white and gray rami of the frog's 7th nerve consist almost exclusively of these typical islands of myelinated and unmyelinated fibers, and Gasser and Erlanger (personal communication) have observed *B* and *C* waves in a gray ramus. Certain dorsal roots of bullfrog and turtle also have similar thinly myelinated axons which are being studied as a possible source of sympathetic outflow. The sympathetic trunk of the frog has besides these, some larger myelinated axons, as has that of the dog. The latter has been found to give a very low potential corresponding in properties to the late *A* group, a higher one corresponding to the *B*. A human genitofemoral nerve gave a large second process following 4 waves of the first process; the cross section shows many typical islands of myelinated and unmyelinated axons, and the refractory phase was short for the first group and long for the second. Failure of the third group to appear from this nerve may have been due to its long time out of the body. The refractory phases of the frog and turtle sciatic Band C waves, like those of the sympathetic, are 4 times as long as that of the A group, as if these waves were, in part, at least, due to sympathetic components.

The turtle vagus is similar in cross section and in potential form and in other properties of the axon processes to the sympathetic, except that more large fibers are present, and a larger first group of potentials. In the cat vagus and cervical sympathetic trunk the same situation holds, except that the islands are not so sharply marked off from areas of large fibers; there are relatively more of the latter and relatively fewer unmyelinated. The potential distribution, conduction rates, thresholds, etc., correspond closely to those of the turtle allowing for differences in temperature. As shown by Gasser and Erlanger[3] for peripheral nerves, so also in the sympathetic nerves the relationship between fiber size and conduction rate (Gasser & Erlanger[4]) fails to hold as between one main group of axons and the next. There is a sharp break in character between the first and second groups in the properties discussed above, and much less difference in certain respects between the small myelinated and unmyelinated fibers. The first group of axons conducts through the superior sympathetic ganglion of the turtle in either direction, the second irreversibly, indicating a motor fiber synapsing in this system. Part of the third group axons, at least, also synapse here and are motor and part go through probably having synapsed in other ganglia more centrally. Some at least of the post-ganglionic fibers synapsing with the second group pre-ganglionic are also second group fibers. If the second group axons are the thinly myelinated ones, post-ganglionic myelinated fibers are therefore present. There are many more of these fibers post-ganglionically than pre-ganglionically.

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[3] Gasser, H. S., and Erlanger, J., Proc. Soc. Exp. Biol. and Med., 1929, xxvi, 647.

It is anticipated that such thinly myelinated axons associated with unmyelinated will *contribute to B* and *C* elevations in other nerves where present, and the investigation of their properties is being extended to test this possibility.

The potential analysis of the sympathetic nerves has been undertaken by Heinbecker, the study of the differentiation between types of axons in various nerves is a joint research of Bishop and Heinbecker.

4441

The Action Potential in Fibers of Slow Conduction in Spinal Roots and Somatic Nerves.

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The group of waves, *alpha, beta, gamma* and *delta,* hitherto described as constituting the conducted action potential of mixed somatic nerve[1, 2] are confluent and are produced by groups of fibers conducting at rates ranging in round numbers, in the bullfrog, green frog (at room temperature) and dog (at body temperature), between 40 and 10, 35 and 8 and 80 and 30 m.p.s., respectively. Together they form what may be called the *A* elevation. When the record obtained from these nerves by the cathode ray oscillograph is continued through 200 to 300 σ, instead of stopping after 10-15σ as has been usual hitherto, and higher amplification, 100,000 instead of 8,000, is used, 2 additional and discrete elevations, which, like *A*, may be compound, appear in succession as the stimulation is raised to strengths far beyond those previously employed by us. The second, or elevation *B* travels at the rate of about 5 to 2 m.p.s. in mixed nerves of the bullfrog and green frog and 17 (20 in one case) to 11 m.p.s. in the dog, the rates in the contributing fibers ranging down to about 2 to 1 m.p.s. in the frog and to about 8 m.p.s in the dog. The third or *C* elevation is conducted at the rate of 0.7 to 0.5 in the frog and 1.5 to 0.8 m.p.s. in the dog, the rates in the slowest fibers ranging down to about 0.3 m.p.s. in the frog and to 0.5 m.p.s. in the dog. The 3 elevations, *A, B* and *C*, are propagated at such diverse rates that in less than 1 cm. of conduction they become quite clear one of the other, in this respect differing strikingly from the *alpha-beta-gamma* waves of *A* which are confluent at all distances of conduction. The heights of induction shocks threshold for *A, B* and *C* in the bullfrog, directly measured by the oscillograph, roughly are, to cite a single instance, 3, 7(+) and 38 volts, respectively; for maximum action potentials *B* and *C* in this case required shocks reaching 38 and 80 volts. This is mentioned merely to indicate how much stronger the stimuli must be to elicit *B* and *C* than those ordinarily used to elicit the action potentials hitherto recorded by us. *A, B* and *C* elevations are present also in the action potentials of skin nerves of the bullfrog and of the dog. In some muscle nerves *B* may be absent or insignificant (phrenic of the dog), in others variable (dog) or well developed (frog).

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[4] Gasser, H. S., and Erlanger, J., *Am. J. Physiol.*, 1927, lxxx, 522.

[1] Gasser, H. S., and Erlanger, J., *Am. J. Physiol.*, 1922, lxii, 496.

[2] Erlanger, J., Gasser, H. S., and Bishop, G. H., *Am. J. Physiol.*, 1924, lxx, 624.

In the bullfrog, of the 3 possible sources of the waves of mixed nerve, all of the anterior roots examined contribute *A* elevations, while some contribute fast travelling *C* waves (about 0.8 m.p.s.); posterior roots supply *alpha, beta* and *gamma* (and possibly *delta*) waves to the *A* elevation, and *C* waves slower than those from the anterior root (rate about 0.5 m.p.s.); and finally grey rami contribute *B* and also *C* processes, the latter first recognized, in the sympathetic nerve of the turtle, by Heinbecker. It would seem, therefore, that there are anterior root, posterior root and sympathetic *C* waves in somatic nerves. The posterior root *C* elevations, like the *A* elevation,[3] are conducted, as nearly as can be determined, at the same rate in either direction through the root ganglion; thus far no evidence has been obtained indicating that the fibers concerned make a synaptic junction there. The rate of conduction of a given *C* process seems to be the same in the root as it is in the nerve trunk.

In the cat, posterior roots (7th lumbar and 1st sacral) contribute to somatic nerves *A* and *C* waves, *C*, as well as *A* being regularly present in root preparations; the corresponding anterior roots supply an *A* wave; and the lower grey rami send in a *C* wave and *B*.

It has been shown[4] that to a first approximation the fibers forming the *A* elevation conduct at rates that are proportional to their diameters. If the *B* and *C* fibers obeyed the same rule, their sizes, in the bullfrog, for example, would range in the case of *B* between 2.5 and 0.5μ and in the case of *C* between 0.35 and 0.12μ. But somatic nerves contain no group of myelinated fibers ranging between the limits demanded by *B;* and myelinated fibers small enough in diameter to conduct at the *C* rate do not exist. The wave in the saphenous nerve of the dog, which answers the present description of *B*, originally was labelled *delta*.[5] Because its rate was slower than could be accounted for on the basis of the fiber-size rule it was believed to be formed by the action potentials of unmyelinated fibers. There are reasons for believing that this elevation and the corresponding elevation in other mammalian somatic action potentials is homologous with the *B* elevation of cold-blooded nerve, namely, its position in the action potential picture, its source, and its failure to obey the fiber-size rule. It travels, however, at a relatively faster rate than the *B* of cold-blooded nerve. The *C* fibers contributed by the sympathetic system probably are unmyelinated.[6] And presumably the *C* elevation in the posterior roots is formed by the action potentials of the unmyelinated fibers (and finely medullated fibers) described by Ranson[7] as passing into the Zone of Lissauer in the cat. The nature of the fibers forming the *C* elevation seen in certain of the anterior roots of the frog is undetermined. The obvious failure of *B* and *C* to obey the fiber-size rule that holds for the *A* system forces the conclusion that the rule can hold only in the case of fibers belonging to one and the same system.

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[3] Erlanger, J., Bishop, G. H., and Gasser, H. S., *Am. J. Physiol.*, 1926, lxxviii, 574.

[4] Gasser, H. S., and Erlanger, J., *Am. J. Physiol.*, 1927, lxxx, 522.

[5] Erlanger, J., *Am. J. Physiol.*, 1927, lxxxii, 644.

[6] Heinbecker, P., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 349.

[7] Ranson, S., *J. Comp. Neurol.*, 1914, xxiv, 531.

Iowa Branch.

State University of Iowa, April 25, 1929.

4442

Atrophy of Anuran Tail Muscle During Metamorphosis.

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From the Zoölogical Laboratory, University of Iowa.

The total atrophy of the larval Anuran's tail constitutes one of the most striking changes evidenced during metamorphosis. The general concept held by many workers has been that the growth of the urostyle so impinges on the arteries supplying the tail that a condition of acidosis results in the latter structure due to inadequate circulation. This acidosis then results in the histolysis of the various tissues with resultant total atrophy. In a recent communication, Helff[1] has shown that the urostyle cannot be concerned since normal atrophy occurs following the removal of the larval urostyle before the latter undergoes growth. At the same time Lindeman[2] was able to show that tail integument underwent normal degeneration during metamorphosis when previously transplanted to the back of tadpoles. The present paper describes the similar transplantation of tail muscle to foreign regions to see whether or not normal atrophy would occur during metamorphosis.

All transplantations were made on large *Rana clamitans*' tadpoles. Four types of autoplastic muscle transplants were made, *viz.,* tail muscle to the back, back muscle to the tail, tail muscle to another region on the tail, and back muscle to another region on the back. The technique for all these operations was briefly as follows: A small 2 mm. cube of muscle was first removed from the back or tail, as the case might be. A flap of integument was then laid back from the region to receive the transplant, and the transplant either laid on the muscle beneath or partially inserted in an incision made in the muscle. The flap of skin was then replaced and the grafts allowed to heal for 2 or 3 days following which period the larvae were metamorphosed artificially with desiccated thyroid. The engrafted muscle was then examined at 10, 15 and 21 day intervals thereafter, and the normalcy or degree of atrophy recorded.

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[1] Helff, O. M., *Anat. Rec.,* 1928, xli, 39.

[2] Lindeman, V. F., *Anat. Rec.,* 1928, xli, 39.

Two hundred and twenty successful transplantations were made which appeared firmly attached and normal within 3 days following transplantation. The average amount of atrophy of the various types of grafts is expressed in Table I.

TABLE I.

Type of Transplantation	Number of cases	Ave. reduction in volume due to atrophy		
		10 days	15 days	21 days
		%	%	%
Tail muscle to the back	130	58	86	(100 cases)—100 (30 cases)— 99.2
Back muscle to the tail	60	21	48	54
Tail muscle to other side of tail	15	38	40	49
Back muscle to other side of back	15	27	38	38

The results as shown in the table throw light on several questions. It is evident that the musculature of the back undergoes a normal reduction in volume during metamorphosis while a still greater reduction is experienced when grafted to the tail. This latter result is to be expected in that the graft is surrounded by tail muscle undergoing rapid autolysis. The extremely rapid atrophy of tail muscle grafted to the back, resulting in total obliteration within 21 days cannot well be explained except by assuming that it is especially susceptible to autolysis and atrophy during metamorphosis. The results in this regard strongly indicate that normal autolysis of tail musculature of involuting frog larvae is the result of a specific reaction to blood changes or other metabolic disturbances developed at a certain stage of metamorphosis.

4443

Effect of Castration on the Oviduct in Males and Females of Rana pipiens.*

KERMIT CHRISTENSEN. (Introduced by Emil Witschi.)

From the Zoölogical Laboratory, State University of Iowa.

Rana pipiens is unique among all frog species for the surprisingly well developed oviducts in the male, although in the male they do not undergo the cyclical changes so characteristic of the female. From anterior to posterior, 4 parts are differentiated in the oviduct: a funnel opening, the *ostium tubae;* then a short, straight tube, the *pars recta;* a convoluted, glandular portion, the *pars convoluta;* and lastly, the uterus. The anterior parts of the oviducts of the male are less developed than the posterior parts. This difference distinguishes the oviducts of the male from those of the female.

Oviducts have been found in male toads and in hermaphroditic frogs and toads[1, 2] but in these cases, the existence of the oviduct is clearly dependent upon some development of ovarial structures. This was suggestive therefore of an investigation to discover if the unusual growth of the oviducts in the male leopard frog also is correlated with hermaphroditic tendencies in the development of the male gonad. The embryological study showed, however, that as a rule the testes are typically differentiated before the time of metamorphosis which may even be before the first appearance of the primordia of the oviduct.

The oviducts in young frogs, both males and females, are similar in their gross structure except for the slightly larger size in the females. In microscopic sections, the size variance is somewhat more evident. In second year frogs, the sex differences in the oviducts are rather striking.

It appears then that 2 phases exist: (1) a period of self-differentiation in both sexes, and (2) in the female only, a period under the control of the ovary in which the oviducts enlarge to their final size and become functional. In the male, further development stops.

To test further the effects of the gonads on the oviducts of adult males and females, females were completely spayed and males were completely castrated in 2 series of frogs. A third series were kept as controls.

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* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

[1] Witschi, Emil, *Arch. Entw. Mech.*, 1921, xlviii, 316.

[2] Crew, F. A. E., *J. Genetics*, 1921, xi, 141.

The spayed females, consisting of 50 frogs in all, were kept from 3 to 8 months. Those kept until the end of 3 months showed a noticeable regression in the oviducts especially in the median part of the *pars convoluta* where the small tubules indicated the beginning of a marked degeneration. After 5 months these degenerative changes had included nearly all parts of the *pars convoluta*. The animals at the end of 8 months exhibited degenerative changes in all parts of the oviduct and in their appearance they come to resemble the oviducts in normal female frogs in the second season. A normal sexually mature female control has the oviducts nearly as large as an adult female caught in nature at this time (breeding season).

The castrated males, a total of 36 individuals, were also kept from 3 to 8 months. After being kept for 3 months the oviducts in these frogs showed no changes, and even at the end of 8 months, the castrated males do not vary in the condition of the oviducts from that found in control males.

In both castrated males and spayed females, there is a noticeable regeneration of the fat bodies.

The results obtained in these series of experimental animals confirm the idea that the female oviducts maintain their normal development and undergo their cyclical changes under the influence of the ovary. Since there is such a marked regression in the oviduct of the spayed female and since in the male there seems to exist no relationship between the gonads and the oviduct, it can be reasoned that the oviduct of the male simply represents the state of development which the oviducts may attain in either sex without a gonadal control. While the ovary causes a further growth beyond this condition, it seems that the testis does not exert the slightest influence.

These findings contradict the claim of Bogucki[3] that in frog females *(Rana esculenta* and *Rana fusca)* the growth and functioning of the oviducts are fully independent of the ovary.

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[3] Bogucki, M., *Bull. Acad. Sci. Cracovie*, 1919, 165.

4444

Occurrence of Heat Labile Toxins in Closed Intestinal Loops.

J. T. MC CLINTOCK AND H. H. HINES.

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The report of Williams[1] has stimulated interest in the possibility that heat labile bacterial toxins may play an important rôle in the toxemias of intestinal obstruction and allied disorders. The physical nature of the material obtained from the intestinal tract in these clinical or experimental conditions is such that it is extremely difficult to prepare a sterile filtrate which exhibits anything like the potency of the original material.

Experiments were undertaken to determine whether the fluid from closed intestinal loops of dogs contained appreciable quantities of filterable heat labile toxins. The method of Berkefeld filtration was employed to obtain sterile material for injection into guinea pigs, rabbits and pigeons. A comparison was made of the toxicity of unheated Berkefeld filtrate and fluid heated to 65° C. for one hour and strained through glass wool. Filtration was extremely slow and the quantity of material available from each closed loop usually permitted only one toxicity test to be made.

Intravenous injections of fluids from 7 closed loops were made in rabbits, but because of the frequent complications of intravascular clotting, the results were undecisive. The toxicity of fluids from 13 closed loops was determined for guinea pigs (intraperitoneal administration). In 6 of the 13 cases no definite toxemia occurred with either the heated or unheated fluid, although the maximum amount of available material was used. In 6 cases the administration of the untreated filtrate in amounts of 1.3 to 9 cc. per kilo was fatal to guinea pigs in from 12 to 48 hours. Equivalent amounts of the heated fluid failed to cause marked intoxication or death. In one case both fluids were apparently equally toxic. No difference was noted in the potency of the material from different parts of the intestinal tract or from loops of animals with different survival times. Attempts were made to determine the presence of the exotoxins of *B. welchii* by an injection of the filtrate into the breast muscles of pigeons. With doses of 3 cc. per kilo no intoxication or lesions were found similar to those described by Bull and Pritchett[2] for the exotoxin of *B. welchii*. It is concluded that an unheated Berkefeld filtrate from closed intestinal loops was more toxic for guinea pigs than fluid heated for one hour at 65° C. The possibility that the diminished toxicity of the heated fluid might have been due to mechanical loss of toxins rather than to thermal inactivation has not been excluded.

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[1] Williams, B. W., *Brit. J. Surg.*, 1926, xiv, 295.

[2] Bull and Pritchett, *J. Exp. Med.*, 1917, xxvi, 119.

4445

The Free-martin Effect in Experimental Parabiotic Twins of Triturus torosus.*

E. WITSCHI and H. M. MC CURDY.

From the Zoölogical Laboratory, State University of Iowa.

It is known since the work of Keller and Tandler[1] and Lillie[2] that in heterosexual twins of cattle with fused chorionic membranes the male part exerts a very characteristic influence on the differentiation and the development of the gonads of its female cotwin. This action results in the following distinctive features of the free-martin gonad:

(1) Sterility in consequence of the absence of germ cells.

(2) Reduced or completely suppressed ovarial cortex.

(3) Hypertrophied medulla with more or less pronounced male type of differentiation.

Experiments with amphibians joined in parabiosis at an early embryonic stage were expected to provide possibilities of a detailed analysis of the mechanism of this effect. This hope was rather disappointed, however, when it appeared from the results of Burns'[3] and Witschi's[4] investigations that Amblystoma and Rana behave otherwise than cattle twins and represent two different and new types of reactions. In heterosexual combinations of Amblystoma either one of the sexes may already in the primary sex differentiation assume the absolute leadership, so that pairs of males and pairs of females are obtained exclusively. In frogs, on the other hand,

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* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

[1] Keller, K., and Tandler, J., *Wiener Tierärztl. Wochenschrift, III. Jahrg.*, 1916, 513.

[2] Lillie, F. R., *Science*, 1916, lviii, 611; *J. Exp. Zool.*, 1917, xxiii, 371; *Biol. Bull.*, 1923, xliv, 47.

[3] Burns, R. K., *J. Exp. Zool.*, 1925, xlii, 31.

[4] Witschi, E., *Biol. Bull.*, 1927, lii, 137; *J. Exp. Zool.*, 1927, xlvii, 269.

the two sexes first develop independently. Later on the male becomes predominant and causes a regular sex transformation in the female cotwin. The case of Rana comes closer to the cattle type than the case of Amblystoma, because of the finally established dominance of the male sex, however, it differs from it by the accomplishment of perfect sex inversion in the female cotwin.

A new group of experiments with the Californian salamander, *Triturus torosus,* yielded results more nearly comparable with the conditions in cattle. Of twenty-two pairs of twins, joined in parabiosis at the stage immediately preceding the beginning of muscular movements and raised until close to metamorphosis, one half proved to be unisexual (4 ♀ ♀ and 7 ♂ ♂), while the other half was heterosexual (6♀ ♂ and 5 ♂ ♀). The second group shows a very interesting range of variation which will be described in detail in a paper under preparation. It comes nearer the cattle type than any other case of experimental parabiosis so far recorded. (1) The male, without exception, is the leading part. Its gonads are always larger, often many times larger than those of the cotwin. (2) The gonads of the female contain only a very few scattered germ cells or are completely sterile. In no case do they contain ovocytes or young egg cells, which at this time are abundant in single controls and in the double female twins. (3) The gonads of the female show a tendency to assume male characters. The medullary cords are compact and only in a few places may show some rudiments of ovarial sacs. In four out of the eleven heterosexual twin females a very few spermatogonia (from 1 to 6 per animal) are found. A fifth one possesses a testicular segment of considerable size in the right gonad, while the left side is perfectly sterile. These occurrences indicate that at least in some cases a complete sex inversion might have been the final result.

While thus, in a general way, parabiosis in Triturus produces an effect which closely resembles the observed consequences of chorionic fusion in cattle, there are some particularities to be mentioned, never recorded so far for any other material. (4) The heterosexual twins influence each other *mutually.* Not only the gonads of the female but also the testes of the male contain a much reduced number of germ cells. It is true that in no case has resulted a completely sterile condition in the male, though the histological appearance of the testis is often thoroughly changed by the scarcity of spermatogonia. This is mainly true in those cases which are conspicuous for a luxuriant growth of the medullary cords.

(5) The most interesting feature displayed by the heterosexual

twins is the dislocation of the germ cells. Especially in the females one finds practically all the germ cells outside of the gonads in the mesovarium in the direction of the kidney as well as towards the fat body. In the males the extra regional germ cells concentrate rather in the hilum region of the gonad. The germ cells outside the gonads are more or less reduced in size but show the characteristic multilobate nucleus. The failure of the germ cells to reach their final position is not due to the general conditions of parabiosis as evidenced by the unisexual twins in which extragonadal germ cells may only exceptionally be found. It evidently is caused by an antagonistic reaction between the sexes, resulting in a mutual inhibition of the processes leading to the differentiation of the primordial gonads. Therefore in this experiment we deal with a so far unique case of manifestation of sex-antagonism prior to the morphological formation and differentiation of the gonads.

4446

Effect of Oestrus Upon Achilles Reflex Time.*

R. YORKE HERREN AND H. O. HATERIUS.
(Introduced by Lee Edward Travis.)

From the Psychopathic Hospital and the Zoölogical Laboratory, State University of Iowa.

Several authors have remarked upon the relationship, in mammals, between the functional sex glands and behavior—general[1] and specific.[2] No work has been done on the relationship between the normal functional sex glands and the central nervous system. This report gives the influence of oestrus on the electromyographically determined Achilles reflex time. Eight normal female rats with known sex histories were followed through 18 consecutive cycles, varying from 1 to 4 cycles per individual, and reflex time determinations were taken during the active phase of the sex cycle—oestrus, and during the quiescent phase—dioestrus. Vaginal smears were made daily according to the technique of Long and Evans (1922) to determine the phase of the cycle. Our determination of

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* The expenses for this investigation were defrayed in part by a grant from the National Research Council Committee on Research in Problems of Sex.

[1] Richter, C. P., *Quart. Rev. Biol.*, 1927, ii, 307.

[2] Macht, D. I., and Seago, D. W., *J. Comp. Psychol.*, 1924, iv, 151.

the reflex time for either period was taken as the average of 5 to 7 consecutive jerks. The recording apparatus and technique has been fully described elsewhere[3] and need not be discussed here.

The average reflex time during the dioestrus interval was .0059 sec.; that for oestrus was .0079 sec., with an average difference between the 2 periods of .0019 sec. The average difference, though not large, is, we feel, significant, especially since it is between one-third to one-fourth of the longest reflex times, and, in all instances, nearly 4 times as great as the jerk to jerk variation of one testing. Two typical readings are included:

RAT HR_1

Date	Period	Times				Average Time
3- 8	Oestrus	.0090	.0095	.0095	.0095	.0094
3-11	Dioestrus	.0075	.0075	.0075	.0075	.0075

There are several possible explanations of the decreased rate of reflex responses. One is that the sex gland during oestrus directly or indirectly causes the liberation of a substance which inhibits the passage of the nerve impulse somewhere along the arc. Another is that the impulse set up by the stimulus has greater radiation during oestrus and hence its appearance in the muscle is delayed. A third is that the functioning of the sex gland disproportionately increases higher nerve center activity which in turn depresses lower nerve center activity. The depressed lower center activity expresses itself in the longer reflex times. This explanation is based upon the findings and interpretations of Travis and Dorsey[4, 5] who found that whatever increases higher correlation center activity lengthens the reflex time and whatever depresses the higher center activity shortens the time due to an increasing and decreasing respectively of the inhibitory action of the higher on the lower motor centers.

That the functioning of the sex gland does facilitate higher centers has been demonstrated by its relation to spontaneous activity, maze running, etc. (*Cf.* Richter.[1])

Our work so far has been limited to females because of the ease with which cyclic sexuality fits into the plan of research. So far work on castrates is too inconclusive to report.

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[3] Travis, L. E., *J. Exp. Psychol.*, 1928, ii, 342.

[4] Travis, L. E., and Dorsey, J. M., *Arch. Neurol. and Psychiatry*, 1929, xxi, 613.

[5] Travis, L. E., and Dorsey, J. M., 1929 (MS. to be published in *Arch. Neurol. and Psychiatry*).

4447

Experimental Study of Ovariectomy and Transplantation in the Pregnant Albino Rat.*

H. O. HATERIUS AND W. O. NELSON. (Introduced by W. W. Swingle.)

From the Zoölogical Laboratory, State University of Iowa.

The work of Herrick[1] concerning ovariectomy and transplantation in relation to the maintenance of pregnancy in the guinea-pig seems to offer evidence contradictory to that reported by previous workers, among others, Fraenkel,[2] Marshall and Jolly,[3] Dripps,[4] Harris.[5] Their results indicated the necessity of the *corpus luteum* at least during the first half of pregnancy. Herrick, employing virgin ovaries for his grafts, and therefore no *corpora lutea* of pregnancy, concludes that the substance in the ovary responsible for the maintenance of gestation is not confined to the *corpus luteum.*

In view of the apparently contradictory results obtained by Herrick it was thought well to make a more thorough study of this phase of the factors involved in the maintenance of pregnancy and if possible to throw some light upon the true rôle played by the *corpus luteum.* The animal selected for this work was the albino rat, 19 experimental cases serving as the basis for this report.

The exact time of copulation was observed in each instance and the dates of the various stages of pregnancy were calculated therefrom.

The ovaries used as grafts were taken from virgin or from immature females in all except 2 cases, pregnant ovaries being there employed. The implants were made between the abdominal rectus muscles in a small pocket. Transplantations were made at different stages of pregnancy (9th, 12th, and 13th days) and extirpation of the host's ovaries was performed in all cases on the 13th or 14th day. A small proportion of the grafts failed to take (3 out of 20), in which event resorption or absorption ensued. This failure was demonstrated by a careful autopsy revealing complete fibrous infil-

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* The expenses for this investigation were defrayed by a grant from the National Research Council Committee for Research in Problems of Sex.

[1] Herrick, E. H., *Anat. Rec.*, 1928, xxxix, 1.

[2] Fraenkel, L., *Arch. f. Gynaek.*, 1903, lxviii, 438.

[3] Marshall, F. H. A., and Jolly, W. A., *Proc. Roy. Soc. London*, 1905, lxxvi, 395.

[4] Dripps, D., *Am. J. Anat.*, 1919, xxv, 117.

[5] Harris, R. G., *Anat. Rec.*, 1927, xxxvii, 83.

tration on the site of the graft. In cases of successful takes, where but a day was allowed for establishment of the graft before removal of the host's own ovaries, pregnancy continued to term. Either the foetuses were still-born or died shortly after delivery. If 4 or 5 days' time was allowed between implantation and ovariectomy normal young were born.

A histological study of transplanted ovarian tissue recovered a day before or immediately following parturition gave evidence of typical lutein tissue. Control ovaries (*i. e.,* the corresponding ovary to the one implanted) showed only immature follicles, no lutein tissue being present.

Five bilaterally ovariectomized females aborted within 2 days following operation. The second ovary was removed on the 14th day of gestation in these controls.

The above data seem to indicate that the *corpus luteum* is essential for the maintenance of pregnancy in the albino rat and that when sufficient time is allowed for vascularization of grafts, immature ovaries transplanted into a pregnant female undergo an adaptation, develop masses of luteal tissue (clearly demonstrable in histological section), and suffice as functional substitutes for the original ovaries in pregnancy.

4448

Differential Reaction of Ovariectomized Pregnant Rats to Ovarian Grafts in Various Stages of the Oestrous Cycle.*

W. O. NELSON AND H. O. HATERIUS. (Introduced by W. W. Swingle.)

From the Zoölogical Laboratory, State University of Iowa.

In connection with a study of transplantation of ovaries removed from virgin rats, into ovariectomized pregnant females it was discovered that a differential effect resulted from the employment of ovaries whose donors were in various stages of the oestrous cycle. Fourteen experimental animals furnish the basis for the following report.

All transplants were made on the 12th or 13th day of gestation, with ovariectomy following within 24 hours. Transplants were placed in specially prepared pockets between the abdominal rectus

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* The expenses for this investigation were defrayed by a grant from the National Research Council Committee for Research in Problems of Sex.

muscles. A complete check upon the oestrous cycle of all donors was made by means of the vaginal smear method outlined by Long and Evans (1922).[1] Only virgin females in full vigor were employed.

Pro-oestrous stage: Five implantations were made of ovaries from rats in pro-oestrous. Abortion occurred in each instance on the average of 75 hours following removal of the host's own ovaries. No cases of resorption occurred, although termination of pregnancy took place on the 16th or 17th day.

Oestrous stage: Two grafts were made of ovaries removed during the oestrous phase. One case of resorption resulted (subsequently found to be due to failure of the graft to take), whereas the second case terminated in abortion on the 20th day.

Met-oestrous stage: Two operations involving transplantation of ovarian tissue from donors in met-oestrous resulted in abortion on the 20th day.

Dioestrous stage: A series of 5 successful implants was made, utilizing gonads from donors in dioestrous. In one of these cases abortion occurred on the 20th day; the remaining 4 carried to term. The foetuses were alive at birth but were not viable, in all but one instance dying within 24 hours.

The results obtained would indicate that the stage of the oestrous cycle of the donor at the time of transplantation may exercise some effect upon the duration of pregnancy in the host in that the time interval between ovariectomy and abortion varies with the cyclic condition of the graft. The fact that abortions occur most quickly when the host is carrying an ovary of pro-oestrous suggests the possibility that the follicular hormone present in the mature follicles of this stage may have some influence in terminating pregnancy; the reaction further indicates a probable rôle which this hormone may play in normal parturition. Conversely, the fact that luteal tissue occurs in its greatest prominence during the dioestrous interval may be of significance in connection with the observation that implants of this stage met with the highest degree of success in the maintenance of pregnancy.

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[1] Long, J. A., and Evans, H. M., *Memoirs of Univ. of Calif.*, 1922, 6.

4449

Some Effects of Insolation on Mineral Nutrition of Triticum.

W. F. LOEHWING.

From the Department of Botany, State University of Iowa.

In an earlier investigation of certain crop plants grown on colloidal humus soils, foliar chlorosis attributable to a physiological insufficiency of iron was reported. Insufficiency of mobile iron and concomitant chlorosis were found to be especially marked in plants exposed to strong insolation. The purpose of the investigation herein reported was to determine if changes in sap acidity induced by light were of sufficient magnitude to reduce the iron supply to the point of chlorosis.

Wheat seedlings for this purpose were grown in an acid humus low in iron. A portion of the cultures were treated with 2000 parts per million of powdered calcium carbonate to correct the known acidity of the soil. Seedlings were grown in glazed pots in a greenhouse at 60° C. until 6 weeks old, at which time analyses were begun. The cultures were evenly divided and placed in adjacent benches subjected to different illumination, one being exposed to full insolation and the other shaded from direct afternoon sunlight by a thin, white muslin screen. Entire plants, freed from soil, were employed and the sap extracted under constant pressure from frozen samples of uniform weight. Acidity of freshly expressed sap was determined potentiometrically in duplicate at 25° C.

The initial analyses, made at 4 hour intervals, disclosed a diurnal cycle of sap acidity which reached a maximum in the early morning and fell steadily to a minimum in the late afternoon in all young wheat cultures. There was some fluctuation from day to day in the hour and amount of the maximal and minimal acidity but the rhythmic diurnal rise and fall continued until the end of the experiment, at which time the plants were 12 weeks old. The variation in time and amount of the afternoon minima was, however, consistently greater than the variation in the morning maxima. Hence the diurnal cycle of sap acidity may be graphically represented as a V-shaped curve whose left limb represents the fall from the morning maximum and whose right limb denotes the subsequent nocturnal rise. The steepness of slope is regulated primarily by the hour and level of the afternoon minimum acidity.

The fact that reversals in direction of the acidity gradients occur approximately at those hours of the day marking the diurnal balance between photosynthetic and respiratory processes within the plant suggest that the light stimulus may be more important than the thermal stimulus of insolation. Though no attempt was made to measure foliar temperatures, spectral composition or thermal energy of the light, it is certain that thermal maxima of the plants were reached much earlier in the afternoons than the maximum pH of the sap. It is very probable, however, that the thermal effect of insolation is in part responsible for the results observed but the precise effect of temperature on sap hydrion concentration remains a subject for further study.

Though illumination appeared uniformly to induce a decrease in sap acidity as described above, differences in light intensity and soil alkalinity exercised considerable influence upon the degree of acidity attained. Limed cultures in the shaded and unshaded sets maintained a lower level of acidity and exhibited a greater fall in sap hydrion concentration in the presence of light than the corresponding untreated cultures. In fact, the pH values for the limed cultures exposed to a light period of maximal insolation on several consecutive days suggest that the period of darkness is too brief for complete acid recovery. Hence acidity falls progressively to a lower level and shows a smaller diurnal fluctuation during protracted periods of intense illumination. Untreated cultures developed chlorosis when strong illumination was used during the normal light period for several days, but the symptoms were less pronounced and less persistent than in alkalinated cultures. Microchemical inspection of chlorotic plants disclosed an abundance of iron in the roots but none in young leaves. Strong insolation thus depressed sap acidity to the point of iron precipitation and ultimate inavailability as evidenced by the chlorotic foliage.

A number of investigators have employed potash with considerable success to correct injury attributable to the use of lime on nutrient deficient soils. To determine the effect of potash in the present instance a dilute solution of KCl was slowly supplied to the limed cultures until potash reached the concentration of 200 parts per million in the soil. The initial effect of this treatment was to stimulate vegetative activity, to raise the general acid level of the sap and to diminish its diurnal fluctuation. Young leaves gradually developed a distinctly green coloration and within 10 days after the initial potash supplement most symptoms of chlorosis had disappeared. The character of response to potash indicates that its influence upon the plants is in part ascribable to increased availability of the salt itself and in part to the resultant increase in tissue fluid acidity which again makes iron available.

New York Meeting.

Yale University, May 25, 1929.

4450

Lesions in Nasal Mucous Membranes of Monkeys with Acute Poliomyelitis.

FRED W. STEWART AND C. P. RHOADS. (Introduced by Simon Flexner.)

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

The ease with which monkeys may be infected with poliomyelitis by nasal instillations of virus either with or without preliminary scarifications of the nasal mucosa has been known since the early experiments of Flexner and Lewis.[1] Moreover, Flexner, Clark and Fraser,[2] and others have demonstrated virus in the nasal secretions of human "carriers". Osgood and Lucas[3, 4] showed the presence of virus in monkeys after the acute stage of poliomyelitis and reported finding virus in the nasal secretion of a human "carrier" 4 months after an acute attack. Kling and Petterson[5] examined the nasal mucosae of monkeys with acute poliomyelitis and observed only acute and chronic inflammation with necrosis. No specific lesions were found.

In view of these facts, it occurred to us to examine histologically the nasal mucous membranes of monkeys dying of acute poliomyelitis to see if any demonstrable changes were evident. All the animals were inoculated intracerebrally and were etherized at various stages of the disease. We were particularly interested in nerve plexuses and ganglion cells within the nasal glands. So far, however, in a series of 31 diseased monkeys, no changes have been encountered in these structures. Chronic inflammatory lesions of mucous glands, infiltration of connective tissues by leucocytes, and leucocytes within the epithelium are not uncommon; one animal showed a fairly well-marked osteochondritis. It must therefore be stated that no lesions suggestive of any specific character were seen in the deeper structures of the nose. Nevertheless, in a certain number of animals the nasal mucosae themselves showed features of considerable interest. 17 of the 31 monkeys with acute poliomyelitis exhibited these lesions to a greater or lesser extent, whereas 9 control animals so far available for study have not yielded similar pictures. Briefly stated, the lesions noted consisted of isolated necroses of the epithelial cells of the respiratory mucosa. It could not be shown that the necrosis was specific for the olfactory receptor-transmittor apparatus for occasional lesions were apparently present in mucus-producing cells. The cell necroses were both superficial and deep. When superficial there was a tendency toward sloughing and the cells were encountered lying free in accumulated mucus. When deep in the epithelium no definite reactional process was apparent.

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[1] Flexner, Simon, and Lewis, Paul A., *J. Am. Med. Assn.*, 1910, liv, 1140.

[2] Flexner, Simon, Clark, Paul F., and Fraser, Francis R., *Ibid.*, 1913, lx, 201.

[3] Osgood, Robert B., and Lucas, William P., *Ibid.*, 1911, lvi, 495.

[4] Lucas, William P., and Osgood, Robert B., *Ibid.*, 1913, lx, 1611.

[5] Investigations on Epidemic Infantile Paralysis. Report from the State Medical Institute of Sweden to the XV International Congress on Hygiene and Demography, Washington, 1912. Published by the Swedish Medical Institute, Nordiska Bokhandeler, Stockholm.

The intracellular lesion consisted of an acidophilic degeneration of the nuclear chromatin, leading to an appearance strongly suggesting inclusion bodies. The nuclei were clear, the chromatin arranged in a characteristic peripheral ring formation rather thicker and coarser than that which we have seen in cells containing the typical inclusions of other virus diseases. The central acidophilic material was single, round, or ovoid, in the form of several smaller acidophilic masses, or more irregularly fused in strands; the single acidophilic body was the most frequent finding. In cells containing these acidophilic bodies the nucleolus was occasionally detectable as a portion of the peripheral basophilic chromatin ring. No finer structure, such as further division of the acidophilic mass giving the well-known mulberry appearance to certain other inclusions, has been observed. The nature of these inclusion-like nuclear bodies is uncertain. The fact that the lesion is not constant in all of the infected monkeys, the fact that the punctate cell necroses are in scattered isolated cells may plead against a specific inclusion idea. Further study is required to determine whether these intranuclear acidophilic bodies are inclusions in a specific sense.

4451

The Monocytic Reaction in Virus III Disease.

LOUISE PEARCE AND A. E. CASEY.

From the Laboratories of The Rockefeller Institute for Medical Research, New York City.

A study of the circulating blood cells has been made on normal rabbits and on rabbits inoculated with various disease-producing agents including virus III. This filterable virus, indigenous to rabbits, causes well marked lesions of the testicle, skin, and scarified cornea when injected at these sites. In the experiments with this virus, the blood was studied at frequent intervals before and after inoculation by means of quantitative determinations of the red and white cells with standardized pipettes, hemoglobin estimations (Newcomber haemoglobinometer), and differential white cell counts of the fresh blood with the supravital technic (vital neutral red).

Three experiments have been carried out. Fresh testicular tissue showing a well developed virus III lesion was ground with sand and normal saline under aseptic conditions and 0.2 or 0.3 cc. of a thick emulsion was injected into one testicle or intracutaneously in the shaved skin of the side of the body. The first experiments comprised 5 normal rabbits which were inoculated intracutaneously and in one testicle; the second series of 5 normal rabbits were inoculated intracutaneously; in the third group, 8 rabbits which had previously been inoculated with a malignant tumor were injected intracutaneously.

The outstanding feature of the observations made was a very marked increase in the number of circulating monocytes, as is shown in the accompanying graph (Fig. 1), which illustrates the findings in the first experiment. In the other series, the results were of the same order but were somewhat less marked, due presumably to the less pronounced reaction associated with the intracutaneous as contrasted with the intratesticular route of inoculation. The results, considered upon a group basis, are recorded as percentage variations from the mean values of 4 counts taken during the week preceding inoculation.

The increase of monocytes, amounting in this instance to 312%, was observed on the fifth day after inoculation at a time when the lesions were at their height of development and when a febrile reaction was present. On the 8th and 9th day, the count had returned to its pre-inoculation level and by this time the lesions had regressed and were practically healed. In the second and third experiments, the corresponding monocytic increase was 234 and 243% respectively, and in both instances the maximum occurred 5 days after inoculation. Observations were not made on the fourth day in the first and second experiments, but in the third series the counts on the second and fourth days were similar and the monocytic rise occurred abruptly on the fifth day.

An increase of circulating large mononuclear cells has been ob-

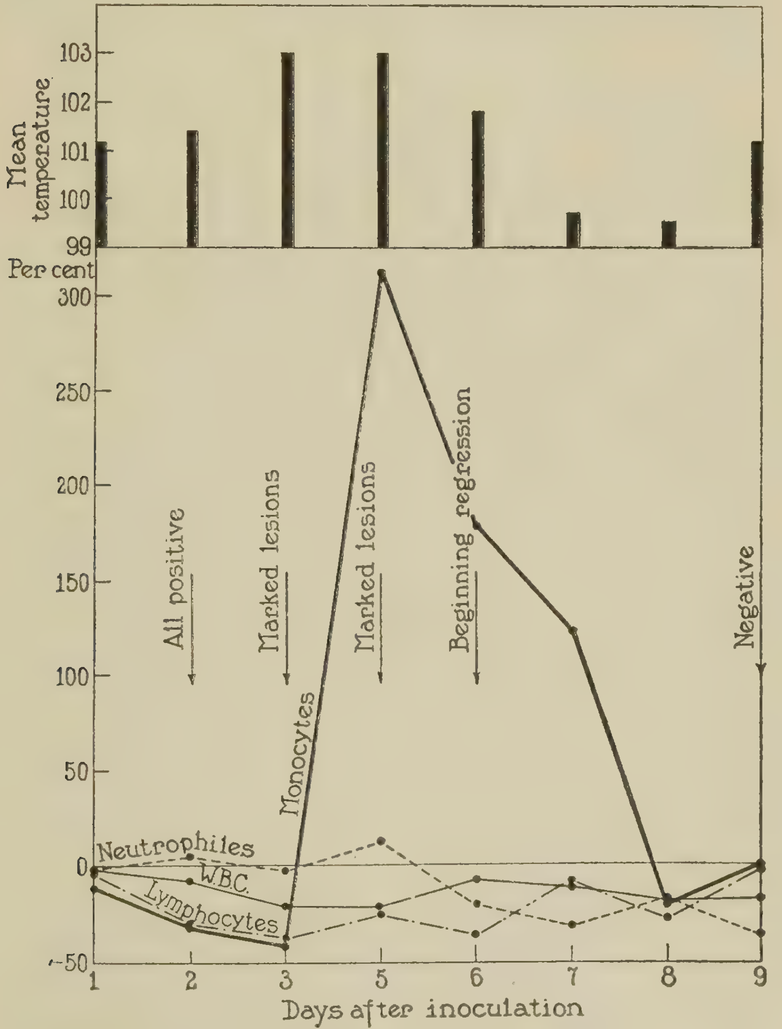

FIG. 1.

Monocytic Reaction in Virus III Disease. Percentage variations from the mean values before inoculation.

served in various diseases, as for example, in tuberculosis, measles, syphilis, vaccinia, and small pox. There is evidence to indicate that in some, if not in the majority of these cases, examination of the fresh blood by the supravital technic would show that many of the cells classified as large mononuclears are monocytes. If this be true, it is reasonable to assume that some feature of the disease process common to these diverse conditions is associated with or responsible for the monocytosis, and the suggestion is here made that a proliferative phase of the general reaction may be the feature in question.

4452

The Monocytic Reaction in Neoplasia of the Rabbit.

LOUISE PEARCE AND A. E. CASEY.

From the Laboratories of The Rockefeller Institute for Medical Research.

A study has been made of the cells of the circulating blood of normal rabbits and of rabbits inoculated with various disease-producing agents[1, 2] including a transplantable malignant neoplasm[3] believed to be of epithelial origin. The results concerning the monocytes in this condition are referred to in the present report.

A variable number of blood counts were taken preceding inoculation and thereafter for 2 months, at which time the experiments were arbitrarily terminated by killing all surviving animals. For the most part, the blood was examined at weekly intervals; a differential white cell count with the supravital technic (vital neutral red) was made at each examination. Six experiments comprising 47 rabbits have been carried out from October, 1927, to March, 1929. In each experiment, a suspension of fresh tumor tissue in normal saline was injected in one testicle of young adult male animals. The course of the disease was followed clinically and the autopsy findings furnished further information concerning the extent and degree of the disease process.

The blood observations have been analyzed with respect to the clinical and postmortem findings and in the present instance, are considered from a group standpoint. The following classification

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[1] Pearce, L., and Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 666.

[2] Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 670.

[3] Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1923, xxxvii, 601.

of animals has been employed: deaths, probable deaths, and recoveries. The first group includes examples of widespread metastatic involvement; the probable deaths comprise those rabbits in which the site and character of the growth found at autopsy 2 months after inoculation were such as to make it appear probable that death would ultimately have resulted from tumor; the recoveries include those animals in which healing of tumor had occurred and in addition, a few instances of slight residual tumor, the nature or location of which was considered as not constituting a probable cause of death.

An outstanding feature of the study of the blood in the 6 experiments so far carried out was a pronounced increase in the number of monocytes in those rabbits which died with more or less widespread distribution of tumor. The findings in one experiment are shown in the accompanying graph (Fig. 1), in which the results are expressed as percentage deviations from the figure 1021, the mean value of the monocytic counts in 1045 observations of normal male rabbits obtained in this laboratory. It will also be noted that in the group designated as probable deaths, the monocytes eventually rose to high levels. In the recovered animals, on the other hand, the fluctuations of the monocytes before and after inoculation were of approximately the same magnitude, and at no time approached the high levels observed in the deaths and probable death groups. The findings in all 6 experiments were of an essentially

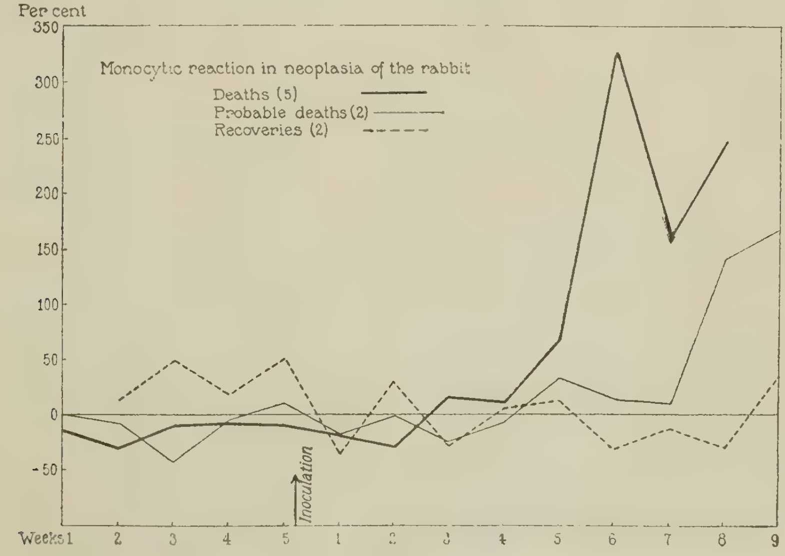

FIG. 1.

similar nature, but it should be stated that in an occasional individual animal dying from tumor, a monocytic rise was not observed. It is possible that this feature would have been revealed by more frequent examination of the blood or that certain rabbits are incapable of exhibiting this reaction. These few exceptions, however, do not affect the group results.

4453

Basophilia as an Index of Resistance.

A. E. CASEY. (Introduced by L. Pearce.)

From the Laboratories of The Rockefeller Institute for Medical Research.

In a recent study of the blood cytology of rabbits[1] which included differential white cell counts with the supravital technic, some observations were made which suggested that the absolute numbers of basophils in the circulating blood might have some relationship to the character of the reaction to infection with *Treponema pallidum*. In general, it was found that those animals which had the highest preinoculation basophil values developed the mildest syphilis and those animals which had the lowest pre-inoculation basophil values developed the most severe syphilis, as determined by clinical examination. The basophils as well as other cells of the blood have also been studied during the course of the disease, and the result of this investigation will be reported later.

Observations were made on 5 groups of male rabbits, a total of 39 animals. The experiments were done at different periods during 1927-28. The blood was examined a variable number of times before inoculation with *T. pallidum*, the number of counts ranging from 4 to 36 per animal per group. The average number of basophils in these counts determined the pre-inoculation level for each animal. The lapse of time between the blood counts and inoculation also varied; in 3 groups the cells were counted during the 3 weeks immediately preceding inoculation, in the fourth group more than a month before inoculation, and in the fifth group the counts were made during a 9 months' period which ended 3 months before inoculation. In each experiment, a tissue emulsion containing actively motile spirochetes was injected in the right testicle. The course of disease was followed systematically over a period of 3 to 4 months.

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[1] Pearce, L., and Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 668.

The relation found between the values for basophils before inoculation and the reaction to the syphilitic infection may be illustrated by a simple comparison of the results obtained with respect to the incubation time of the primary lesion and the metastatic orchitis, and by the occurrence and non-occurrence of such disease manifestations as edema of the inoculated testicle, metastatic orchitis, and generalized lesions.

All animals developed a primary orchitis. In 4 instances, the primary and metastatic orchitis were delayed more than 10 days beyond the average time for the group; 31 animals developed a metastatic orchitis and 6 did not (2 animals were without left testicles); an edema of the inoculated testicle occurred in 13 but not in 26 animals; generalized lesions developed in 24 but not in 14 rabbits (1 animal died of a concomitant infection). The character of the generalized lesions was as follows: bone, with or without skin, 20 animals; skin, with or without bone, 13 animals.

An analysis of the pre-inoculation basophil counts from the standpoint of these features of the disease (Table I) shows the following relations:

TABLE I.

Modes of the Pre-Inoculation Basophil Counts Classified According to Certain Features of the Syphilitic Infection.

Group	No. of rabbits	Mode	Group	No. of rabbits	Mode
		per cmm.			per cmm.
Delayed incubation period	4	2093	No delay	35	875
Edema of inoculated testicle	13	1100	No edema	26	750
No metastatic orchitis	6	1375	Metastatic orchitis	31	875
No generalized les'ns	14	1125	Generalized lesions	34	750
No bone lesions	18	1375	Bone lesions	20	750

The results have also been analyzed from the inverse point of view as shown in Table II. The value 972 used in this comparison represents the average number of basophils per cm. of blood as derived from 1045 counts upon normal male rabbits.

From the data so far collected, it can not be predicated that the apparent relation of a low basophil count preceding inoculation and severe syphilitic manifestations, or *vice versa,* are fixed relations or that they will hold under all circumstances. But if a delay in the development of orchitis, the absence of metastatic orchitis, the occurrence of edema, and the absence of generalized lesions are indices of resistance to infection with *T. pallidum,* then it would seem that

TABLE II.
Pre-Inoculation Basophil Values and Events in the Course of the Syphilitic Infection.

Events	19 animals with basophils above 972 per cmm.	20 animals with basophils below 972 per cmm.
	%	%
Primary orchitis	100.0	100.0
Delayed incubation period	21.0	5.0
Edema in inoculated testicle	47.0	20.0
Metastatic orchitis	65.0	95.0
Generalized lesions	47.0	79.0
Bone lesions	26.0	79.0
Skin lesions	37.0	32.0
Number of generalized lesions per animal	1.46	4.47

the pre-inoculation basophil count might be used as a rough index of the potential reaction of the rabbit to syphilis. It has been found, however, that there are striking exceptions to this rule when applied to individual animals, and at present, the extent to which such exceptions may affect group results is not entirely clear. An analysis of the material from the standpoint of the relation of basophils to other cells of the blood may clarify this situation.

4454

Diagnostic Significance of Precipitin Tests With Anderson Phosphatide Fractions from Human, Bovine, and Avian Tubercle Bacilli.

CHARLES A. DOAN.

From the Laboratories of The Rockefeller Institute for Medical Research, N. Y.

Working in cooperation with the Research Committee of the National Tuberculosis Association, Drs. F. R. Sabin, C. E. Forkner, and the writer, in this laboratory, have been assaying biologically during the past 3 years the various chemical fractions obtained from the tubercle bacillus by Drs. T. B. Johnson and R. J. Anderson of the Sterling Chemistry Laboratory, Yale University. The investigation includes a parallel study of the chemistry and biology of several members of the acid-fast group of bacteria grown in large quantities by Mulford and Parke, Davis under standard conditions on Long's synthetic media. It has been found that the phosphatide fraction isolated from the tubercle bacillus by Anderson[1] contains an active principle capable of stimulating and maturing monocytes and epithelioid cells.[2] This phosphatide fraction from the human tubercle bacillus H-37 has been shown by Pinner[3] to act as a complete antigen when given intravenously in animals, as proven by precipitin and complement fixation tests. We have confirmed this finding with reference to the precipitin reaction. Readings are made after 5 minutes at room temperature, after 2 hours at 38° C., and after 24 hours in the ice-box. Pseudo-positive reactions are thus avoided. Considerable experience in reading the tests is necessary for accurate determinations. Variations in hydrogen ion concentration, pH 2.5 to 6.02, have had no influence on the reaction.

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[1] Anderson, R. J., *J. Biol. Chem.*, 1927, lxxiv, 537.

The sera of normal rabbits have been found to give a positive precipitin test in low dilutions only, 1-20 with dilutions of a 1% antigen, or with a fixed antigen with only the undiluted serum. The exceptional rabbit direct from stock showing higher titres is now discarded for all experimental work in fraction testing and other studies on tuberculosis in this laboratory. There is some evidence that small variations in the phosphatide precipitin titre may accompany a non-tuberculous monocytosis in blood and connective tissues.

In 36 rabbits given from 3 to 8 antigenic doses of 10 to 25 mgm. each of phosphatide A-3 from H-37 human strain of tubercle bacilli, either alone or combined with horse serum, the rabbit sera on the 12th day after the last dose have shown precipitin titres with diluted antigen of from 1-160 to 1-450. Dilutions of sera against a fixed antigen (1-10) have been positive in 1-30 to 1-100. The higher titres have been obtained when the phosphatide was given in combination with horse serum and in the larger dosage. Specific absorption with the homologous antigen has rendered these sera inactive.

The sera from tuberculous rabbits have been positive in degree directly proportional to the dosage of organisms and extent of lesions (0.01 to 2 mgm. B-1 intravenously). An increase in the titre of the blood serum may be noted as early as the third day after inoculation when a dosage of 0.5 mgm. or greater has been given. During the first 2 weeks particularly, testing for antigen with anti-phosphatide rabbit sera gives positive precipitin tests with the sera from tuberculous animals diluted up to 1-40. The anti-phosphatide serum is kept in sterile sealed ampoules and used as needed. In rabbits with chronic generalized tuberculosis and involvement of the central nervous system both blood serum and ventricular fluid show strongly positive precipitin tests. Although the phosphatides from human, bovine and avian bacilli all react with bovine tuberculous rabbit sera, it has been our experience thus far that the bovine phosphatide tends to give a more massive precipitate in corresponding dilutions and to react in slightly higher dilutions than do the other 2, the differential reaction being quantitative rather than qualitative. Lecithin, phospholipin from brain tissue (Dr. P. A. Levene), and resorcinol, the substance used in the Verne flocculation test, give positive readings with tuberculous rabbit sera but when run in dilutions parallelling the phosphatides from the tubercle bacillus, they usually cease to react in relatively low dilutions. Old Tuberculin, protein, 304-B from the bacillus (Dr. T. B. Johnson), 304-F from the filtrates (Long and Seibert), and the polysaccharide (Anderson) from H-37 human tubercle bacilli always give precipitin reactions in well advanced stages of the disease, but in rather lower titre than the phosphatide and with less consistency. It may be that when similar fractions are available from the bovine organism more consistent results will be obtained in the bovine tuberculosis of rabbits.

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[2] Sabin, F. R., and Doan, C. A., *J. Exp. Med.*, 1927, xlvi, 645.

[3] Pinner, M., *Am. Rev. Tuber.*, 1928, xviii, 497.

The blood sera from chicken and guinea pig with avian tuberculosis (Dr. L'Esperance) have shown positive reactions with the homologous antigen in titre of 1-320, while the titre with human and bovine phosphatide has been 1-160.

We have received from Drs. Cotton and Woolfolk of the Bureau of Animal Industry, U. S. Dept. of Agriculture, blood sera from 10 tuberculous cows, condemned tuberculin reactors with lesions proved at post mortem. These sera have reacted positively with the precipitin test, bovine phosphatide being used as antigen, in titres ranging from 1-640 to 1-10,000, The human and avian phosphatides have given less massive precipitates and in somewhat less dilution. The sera from 10 non-tuberculous cows were furnished by Dr. Little, of the Princeton branch of the Rockefeller Institute, none of which showed a titre above 1-80. It has been noted that certain of the tuberculous sera show a faint cloud at the juncture between serum and fresh glass distilled water at the 5 minute reading, which has not been seen with non-tuberculous sera. This reaction later, however, is minimal and permits differentiation between positive and pseudo-positive readings.

Absorption experiments with equivalent weights of the various phosphatides and the tuberculous cattle sera showed a distinctly larger quantity of precipitate when the bovine material was used than with either human or avian, and subsequent precipitin tests showed corresponding degrees of absorption. Human tuberculous blood sera and pleural effusions giving strongly positive reactions with the phosphatide fractions have been rendered non-reactive after absorption with the homologous phosphatide.

Through the interest and courtesy of Dr. E. A. Park of the Harriet Lane Home, Johns Hopkins Hospital, Baltimore, the beginning of a clinical study with these chemical fractions was suggested and has been made possible. Because of the many disappointing failures in the past with precipitin tests, a considerable experience with the newer fractions now available must accumulate before critical judgments may be formed. It has seemed worth while to repeat some of the earlier work along these lines both because of the standard bacteriological and chemical technique that is now being used in these cooperative studies and because of the eventual availability of parallel fractions from different members of the acid-fast group of bacteria for comparison and contrast. We are greatly indebted to a number of clinics and sanatoria for the fluids tested and the clinical data.*

We have tested thus far blood sera, spinal fluids, pleural effusions, ascitic fluids, or joint fluids from 134 individuals. Of 12 cases of tuberculous meningitis in infants, only one has failed to give precipitin tests either with the phosphatide, or with the anti-phosphatide rabbit sera. The latter has given promise of being the most sensitive test in early cases and in those apparently unable to react to the antigen of the bacillus. Whereas normal rabbit serum is entirely inert, the sera from rabbits which have reacted to the phosphatide in antigenic doses give precipitin reactions with dilutions of the tuberculous fluids as high as 1-5000. The "zone phenomenon" is strikingly brought out in these tests. For example, in one tuberculous spinal fluid 1-0, 1-5, and 1-10 dilutions gave ± readings, with maximum precipitate ++++ at 1-40 and 1-80, and final positive + at 1-5000. In one case in which repeated studies of the spinal fluid were made during the course of tuberculous meningitis, the precipitin titre for antibody starting at a positive of 1-20 increased 12 hours later to 1-40 and 4 hours later to 1-160. Sixteen control spinal fluids from infants representing mumps meningitis, influenza, pneumococcus III and IV, and meningococcus meningitis, congenital lues, pertussis, middle ear infection with convulsions, post-influenzal hemorrhagic encephalitis, and lethargic encephalitis have all been negative with the exception of one case. With a positive precipitin reaction of 1-160 in blood and spinal fluid this case has shown negative tuberculin tests and is running the course of an epidemic encephalitis. Eight spinal fluids from tuberculous individuals not showing involvement of the central nervous system have been negative, 24 normal spinal fluids from adults, and 3 four plus Wassermann spinal fluids were negative. The spinal fluids from 3 non-tuberculous monkeys with poliomyelitis (Dr. C. P. Rhoads) did not react with the phosphatides. The blood sera from all tuberculous infants have been strongly positive, the sera from young non-tuberculous children seldom giving titres above 1-80. Tuberculous joint fluid has given strongly positive precipitin reactions in one case, in contrast to fluids from joints in acute rheumatic fever. In infants and children with primary infections it may be expected that such precipitin tests as these, if found dependable, will prove of greatest value.

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* Our sincere appreciation for material and cooperation is due Drs. E. A. Park, A. M. Chesney and H. L. Amoss, Johns Hopkins Hospital, Dr. H. C. Smith, Bay View Hospital, Baltimore, Dr. Dorothea M. Moore, New Haven Hospital, Dr. W. H. Morriss, Gaylord Farm Sanatorium, Dr. W. S. McCann, Strong Memorial Hospital, Rochester, N. Y., Dr. M. Wollstein, Babies Hospital, Dr. F. W. Stewart, Memorial Hospital, and the staff of the Rockefeller Hospital and of Bellevue Hospital, New York City.

In adults the application of the principle to serous exudates and fluids of questionable etiology may prove of considerable aid to early diagnosis. The 14 pleural effusions from known tuberculous cases have proven to be strongly positive when tested with phosphatide and have reacted with the protein 304 and the polysaccharide in some instances in comparable titre. A proper evaluation of these findings must await a larger series of cases with non-tuberculous controls. The blood sera from normal adults, 2 with known positive tuberculin skin tests, and from cases of lymphosarcoma, lymphatic leukemia, lethargic encephalitis, gastric carcinoma and pyogenic infections have all given readings under 1-40 or 1-80. Three non-tuberculous ascitic transudates have been negative. In certain suspected cases still being studied and in all well advanced cases of pulmonary tuberculosis the precipitin titre in the blood against a fixed antigen has been 1-80 to 1-240 and with antigenic dilutions 1-400 and above. Tentatively, a titre of 1-160 to 1-320, antigenic dilution, or 1-20 to 1-40 serum dilution may be taken as suggestive, and titres above these as usually indicative of an active process. As in most tests for tuberculosis positive findings are more significant than negative.

Through the cooperation of Dr. Fred W. Stewart the blood sera from 9 cases of Hodgkins disease have been studied. All have given positive precipitin titres of from 1-320 to 1-3000. Further comparative studies are in progress with the above mentioned and other fractions from the various acid fast organisms.

Differential counts of the living supravitally stained cells from freshly drawn fluids other than blood were made in selected cases at the Harriet Lane Home, Johns Hopkins Hospital, Baltimore, through the courtesy of Dr. E. A. Park. For example, complete cellular studies of blood and spinal fluid were made in one infant under 1 year with generalized tuberculosis and meningitis. In the peripheral blood there were 47% neutrophilic leucocytes, 11% lymphocytes, 40% monocytes and 2% epithelioid cells. The ventricular fluid was clear with 450 cells and the centrifuged sediment showed 10% neutrophilic leucocytes, 46% small lymphocytes, 2% intermediate lymphocytes, 6% young monocytes, 20% monocytes, 5% epithelioids, 2% Langhans giant cells, 3% clasmatocytes, 2% serosal cells, 1% neutrophilic myelocytes, and 3% degenerating cells. The 2 giant cells had 5 and 7 nuclei respectively arranged peripherally around a central rosette of neutral red bodies. Other tuberculous spinal fluids have shown similar trends with epithelioid cells present, in contrast to the cellular findings in meningeal irritations of other etiology, as for example pneumococcus meningitis. It is essential that the studies be made within 20 minutes after withdrawal of the fluid to be examined as degenerative changes occur very rapidly rendering identification of individual cells impossible. These observations suggest the importance and possible value of careful studies of the cells in serous exudates and fluids from cases with questionable or obscure diagnoses.

4455

Is the Sensitizing Effect of Cholesterol for the Antigen Used in the Complement-fixation Test for Syphilis Due to the Contaminating Sterol, Ergosterol?

ELIZABETH MALTANER. (Introduced by Augustus Wadsworth.)

From the Division of Laboratories and Research, New York State Department of Health, Albany.

Cholesterol as ordinarily prepared is now known to be contaminated with another sterol of an unsaturated and labile type of which ergosterol is the only known representative. Moreover, it is because of this contaminating sterol, ergosterol, that after irradiation with ultra-violet light, cholesterol acquires antirachitic properties. After removal of ergosterol, cholesterol is inert, following irradiation.

Professor Knudson of the Albany Medical College very kindly offered a supply of relatively pure cholesterol and ergosterol and suggested that a comparative study be made to determine whether the sensitizing action which cholesterol has upon the antigen used in the complement-fixation test for syphilis is also due to the presence of the contaminating sterol, ergosterol.

The antigen used was an alcoholic extract of dried, ether-extracted beef heart tissue. A comparison of the activity of the untreated antigen and of the antigen sensitized with the different samples of cholesterol and ergosterol used was made in a series of complement titrations carried out in the presence of antigen alone, serum alone and combinations of normal serum and antigen and syphilitic serum and antigen. With a few normal and syphilitic sera the relative differences in specific activity of the sensitized and unsensitized antigen were thus made apparent and the results further confirmed by comparative complement-fixation tests of the sera from 44 persons having syphilis and of 9 sera from apparently healthy individuals.

In these experiments, the sensitizing action of a sample of cholesterol which was purified by the dibromide method to remove the contaminating sterol, ergosterol, was found to be practically equal to that of the unpurified sample. Moreover, ergosterol alone in the amounts in which it might be present as a contaminant of cholesterol, namely, 0.0002% to 0.02%, had practically no sensitizing action. It appears, therefore, that the sensitizing action of cholesterol, as ordinarily prepared, on antigens in the complement-fixation test is not due to the contaminating sterol, ergosterol.

However, it was interesting to note that ergosterol in larger amounts, 0.2%, had a definite sensitizing action upon antigen, equal in degree to that of equivalent concentrations of the relatively pure cholesterol. Ergosterol, however, was less soluble than cholesterol and its range of usefulness as a sensitizing agent was therefore more limited.

Irradiation with ultra-violet light had no effect upon the sensitizing activity of either cholesterol or ergosterol.

4456

Congenital Hypersensitiveness Transmitted to the Third Generation.*

BRET RATNER AND HELEN LEE GRUEHL.

From the Departments of Immunology and Pediatrics, University and Bellevue Hospital Medical College, New York University.

In studies on the duration of passive hypersensitiveness we[1] have shown that an animal, passively sensitized *in utero* by passage of antibodies from the mother via the placental route, loses its hypersensitiveness in about 2½ months.

The purpose of the studies undertaken at this time was to determine the duration of active hypersensitiveness which results from sensitization produced in the fetal stage of the guinea pig by passage of antigen via the placental route a few days prior to birth.[2] Thus far we have determined that the animal actively sensitized *in utero* retains its hypersensitiveness for at least 6 months.

On account of the persistence of this active state in the second generation, it is possible for an animal so sensitized to transmit her antibodies to the fetus and thus passively sensitize the offspring of the third generation. These 2 phases of transmission of hypersensitiveness are illustrated by the following family in which we have a pregnant female injected with 11 cc. horse serum 4 days before parturition. The 2 offspring (second generation) were shown to be actively sensitized by the fact that they exhibited fatal anaphylaxis at 109 and 143 days respectively. The third generation was shown to be passively sensitized, for 1 animal died when 3 days old, requiring no incubation period for the establishment of anaphylaxis. The sensitization had definitely worn off, for the second animal of this generation was shown to be negative at 127 days. These criteria have already been established in the previous studies referred to. The 2 animals of the fourth generation were negative at birth.

To summarize, we have a mother guinea pig who receives a sensitizing dose of horse serum a few days before parturition and thus actively sensitizes her offspring via the placenta. The second generation—in which the active state of hypersensitiveness persists—in turn passively transmits a state of hypersensitiveness to its off-

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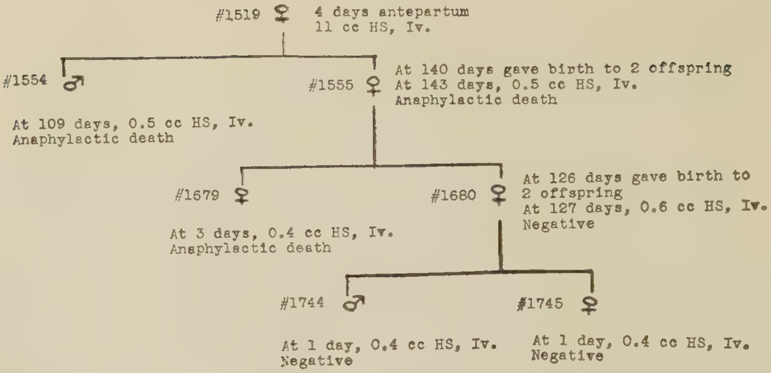
*This work is being carried on under "The Crane Fund for the Study of Allergic Diseases in Children."

[1] Ratner, B., Jackson, H. C., and Gruehl, H. L., *J. Immunol.*, 1927, xiv, 291.

[2] Ratner, B., Jackson, H. C., and Gruehl, H. L., *J. Immunol.*, 1927, xiv, 303.


#1519 ♀ 4 days antepartum
11 cc HS, Iv.
#1554 ♂
At 109 days, 0.5 cc HS, Iv.
Anaphylactic death
#1555 ♀ At 140 days gave birth to 2 offspring
At 143 days, 0.5 cc HS, Iv.
Anaphylactic death
#1679 ♀
At 3 days, 0.4 cc HS, Iv.
Anaphylactic death
#1680 ♀ At 126 days gave birth to 2 offspring
At 127 days, 0.6 cc HS, Iv.
Negative
#1744 ♂
At 1 day, 0.4 cc HS, Iv.
Negative
#1745 ♀
At 1 day, 0.4 cc HS, Iv.
Negative

spring, the third generation. This passive sensitization wears off in the third generation, before the birth of the fourth generation.

Thus the transmission of hypersensitiveness here is *congenital* and either active or passive in nature. When it is passive it can be transmitted only to the succeeding generation, when it is active it may be transmitted through a second generation to a third generation.

This mechanism, we believe, is intimately associated with the problem of the sensitization of the human fetus.[3]

4457

Effect of Calcium and Potassium on Action of Ephedrine.

WILLIAM SALANT AND ELKIN VOGT.

From the Department of Physiology and Pharmacology, University of Georgia, Augusta, Georgia.

The action of ephedrine on the isolated heart was studied by several investigators, among them Chen and Meek,[1] Barlow and Sollman,[2] and Da Costa.[3] The latter also made observations on the influence of calcium and potassium upon the paralyzing action of ephedrine on the heart. It seemed, nevertheless, desirable to repeat these experiments especially for the purpose of using them as a basis of comparison with the effects of ions on the action of this substance. We, therefore, carried out experiments with ephedrine and different amounts of calcium and potassium, and also when either one or both ions were omitted from the solution containing the substance. The experiments were conducted on the isolated frog heart which was perfused by a method that is a modification of the one previously used by the senior author and his collaborators.[4] As it will be published separately by one of us (V), its description is not included in the present report. The results which we obtained with different concentrations of ephedrine in normal Ringer indicate that small amounts, 1-1,000,000 to 1-1,100,000, sometimes increased moderately the force of the contractions. Stronger concentrations, 1-10,000 and especially 1-5,000, greatly decreased the amplitude and to a smaller extent also the rate. Recovery occurred in all cases, neither the duration of the exposure to ephedrine nor the amount of depression which it produced exerting any injurious effect. We noticed, on the contrary, that the contractions were stronger in the after period than in the fore period. By varying the calcium content the following results were obtained with ephedrine. Solutions of 1-100,000 and an excess amount of calcium (0.096%) produced no effect or slight stimulation, the behavior of the heart being the same as when perfused with normal Ringer containing the same amount of ephedrine. The action of larger amounts of ephedrine depended upon the concentration of calcium. When the latter was increased to 8 times the normal amount (0.096%), the depression produced by 1-5,000 ephedrine was abolished. Increase of calcium to 4 times the quantity in normal Ringer still antagonized the action of this amount of ephedrine, but this was not observed when the concentration of calcium was doubled, its action being the same as when perfused with normal Ringer.

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[3] Ratner, B., *Am. J. Dis. Child.*, 1928, xxxvi, 277.

[1] Chen and Meek, *J. Phar. and Exp. Ther.*, 1926, xxviii, 31.

[2] Barlow and Sollman, *J. Phar. and Exp. Ther.*, 1927, xxx, 21.

[3] Da Costa, S. F. G., *Comp. rend. Soc. de biol.*, 1927, xcvi, 1332.

The activity of ephedrine is also modified by reducing the amount of calcium. Stronger solutions (1-5,000) of ephedrine in 0.006% calcium produced much greater depression than the same concentration in normal Ringer. But if the amount of calcium was reduced to 0.003% little or no change was produced by ephedrine. In all experiments with deficient calcium initial stimulation, lasting about 20 seconds, was observed when the heart was perfused with ephedrine, and this was much greater when calcium as well as potassium was reduced to one fourth the normal amount. By removing both calcium and potassium from the solution the action of ephedrine was still more increased, the force of the contractions being augmented several times in one experiment while in another in which the heart was brought to a standstill, contractions appeared promptly when perfusion with 1-5,000 ephedrine was begun and lasted several minutes. It was observed, however, that a repetition of the perfusion with ephedrine was without effect. Weak solutions, 1-1,000,000, also stimulated the heart but this was transitory, and was appreciably less than after perfusion with the stronger solutions. But omission of calcium, which caused the usual effect, produced no change upon adding ephedrine, the heart remaining at a standstill. The results obtained when ephedrine and different amounts of potassium were perfused through the heart were not constant. Depression was produced by a strong solution of ephedrine, the amplitude was moderately decreased and the heart rate was also slower, but it was less affected than the force of the contractions. In some experiments the same amount of ephedrine arrested the heart for a brief period after it was perfused for 20 to 30 seconds with such a solution containing 4 and 8 times the amount of potassium in normal Ringer. The effect in others was marked by pronounced arrhythmia, heart block, and group contractions which occurred when 4 times normal potassium and ephedrine were perfused. It was also observed in this case that heart action was greatly stimulated when this solution was followed by normal Ringer without ephedrine, no such after effect being noticed when heart action was regular during perfusion with such a solution. Experiments were also performed with ephedrine in Ringer solution containing 0.035% or one fourth normal potassium. Ephedrine in this case slowed the heart considerably and greatly decreased the force of the contraction in one experiment. However, this was observed only after the second perfusion, the amplitude after the first perfusion being appreciably increased.

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[4] Salant and Livingston, *Am. J. Physiol.*, 1916, xli, 21.

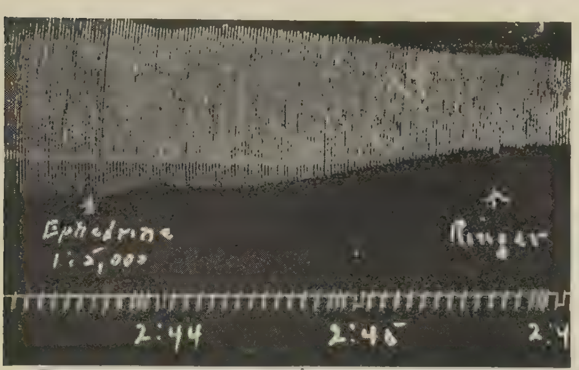
These results led to the further inquiry into the action of ephedrine in excess potassium and calcium. Ephedrine improved heart action also in this case. The contractions, though slower than normal, became regular after 1 or 2 minutes. The beneficial action was further indicated when the same Ringer solution without ephedrine was perfused. Complete heart block alternating with groups of 5 or more contractions occurred and persisted during the perfusion.

It may be added that powdered as well as crystalline ephedrine was used in these experiments. It was sometimes found that the latter was less effective.

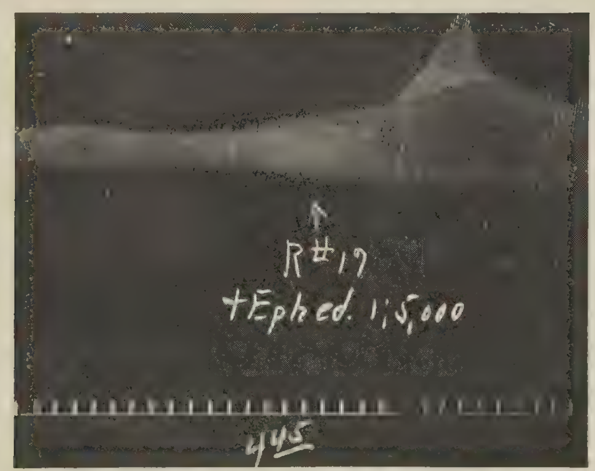
Summary. 1. Small amounts of ephedrine in normal Ringer either increase cardiac activity moderately or are without effect, but a concentration of 1-5,000 always depresses the heart.

2. The depression produced by ephedrine is antagonized by adding sufficient amounts of calcium and aided by reducing the cal-

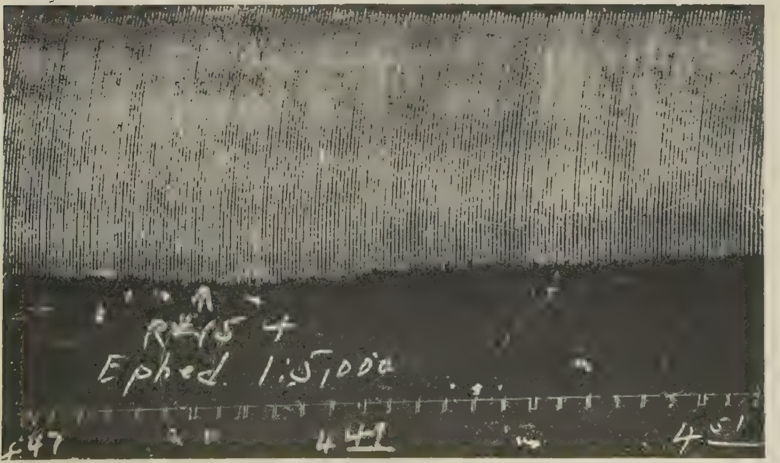
Fig. 1.



A. Ephedrine in normal Ringer.

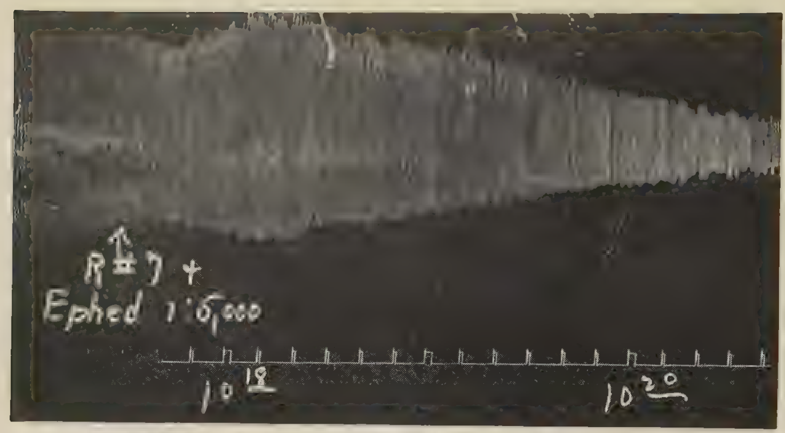


B. Ephedrine in Ringer minus calcium and potassium.

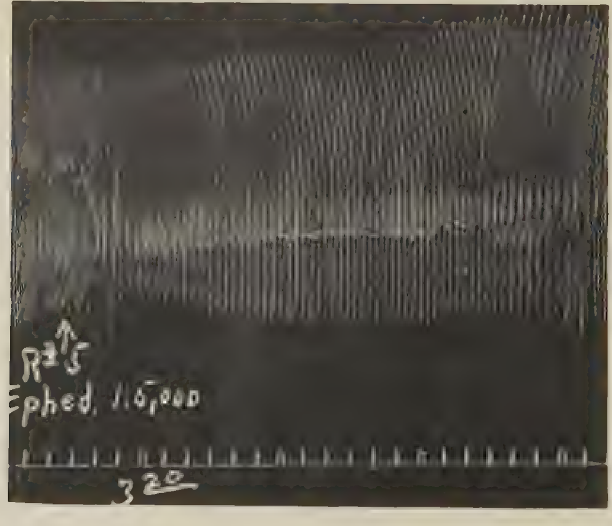


C. Ephedrine in Ringer containing 8 times the amount (0.096%) of calcium in normal Ringer.

Fig. 2.



A. Ephedrine in Ringer containing 0.006% (half normal) calcium.



B. Ephedrine in Ringer containing 0.048% (4 times normal) potassium.

cium content to about 50%. In still smaller amounts of calcium ephedrine is inactive.

3. Ephedrine stimulates the heart when both ions are omitted.
4. Ephedrine in excess potassium depresses the heart but this is much less than in deficient calcium.

4458

Preliminary Quantitative Studies on Action of Follicular Hormone in Spayed Monkeys.

J. A. MORRELL, H. H. POWERS AND J. R. VARLEY.
(Introduced by J. F. Anderson.)

From the Research and Biological Laboratories, E. R. Squibb and Sons, New Brunswick, N. J.

Since the various preparations of follicular hormone, which are commercially available for clinical use, are assayed only in terms of their estrus inducing effect in the white rat or mouse it seems highly desirable to obtain some information, if possible, regarding the quantitative effect of this hormone in spayed monkeys. The studies of Corner, 1923, and Allen, 1927, on the sex cycle of the common *macacus rhesus* monkey paved the way. Allen showed that in some monkeys, in some cases, menstruation could be produced by the injection of the follicular hormone. An attempt is under way at the present time to make a quantitative study of the action of the follicular hormone in monkeys. This is a preliminary report of the work.

Technique: Completely ovariectomized, sexually mature, healthy monkeys of the *macacus rhesus* species were used. The injections were made subcutaneously, using aseptic technique. Sterile, aqueous solutions of the hormone that had been previously standardized by the Allen and Doisy rat test were used. Equal injections were made at 8:00 a. m. and 4:00 p. m. daily for a period of 6 days. Vaginal smears were taken daily and the appearance of red blood cells in the smear taken as the criteria of menstruation.

Every one of our colony of 20 monkeys has menstruated at least once under the influence of injections of follicular hormone, leaving little doubt that if sufficient hormone be injected into a spayed monkey, menstruation will invariably result. Our present data are insufficient to definitely establish the relationship of weight and of the length of the interval since the last menses to dosage, but the indications are that weight is not an important factor. The interval since the last menses may, however, influence the reactivity of the animal.

As may be seen from the table there is apparently no relation between dosage and the amount of epithelial growth, the length of the menses, and the date of the beginning of menses with reference to the date of injection. The color and swelling of the sexual skin was found to be roughly proportional to the dosage for the higher dosages, however.

TABLE I.

Total No. of monkeys injected	Ave. No. of days of epithelial growth	No. of monkeys menstruating	Ave. No. of days of menses	No. of monkeys failing to menstruate	Ave. No. of days after last injection that menses started	Total units injected in each monkey
6	7	3	3 2/3	3	11	30
9	7 8/9	6	5 1/3	3	10 1/6	48
12	8 1/2	10	4 1/2	2	12 2/5	60
7	9 6/7	6	4 1/3	1	10 1/6	72
6	8 2/3	5	5 1/5	1	9 3/5	84
2	6 1/2	2	5 1/2	0	10 1/2	90
3	7 2/3	3	5 1/3	0	9 1/3	96
2	7 1/2	2	4	0	10 1/2	120
2	7	2	5 1/2	0	9 1/2	240

Thus far it is impossible to say just what the minimum effective subcutaneous dose is for a monkey. Failures are reported up to 84 units, but the largest number of failures occurred at 30 units and 48 units. At present we can only say that at 30 unit dosage about 50% of the monkeys responded and no failures occurred where over 84 units were injected.

4459

The Male Hormone: Its Standardization.

JAMES L. MC CARTNEY. (Introduced by A. J. Carlson.)

From the Blackburn Laboratory, St. Elizabeth Hospital, Washington, D. C.

Method of Extraction: The hog testes used was first carefully freed of all extraneous tissue, and then put through a sausage mill. The substance was then thoroughly washed with weakly acid ice-cold water, and filtered, to remove the spermatic element. Half the volume of ether was added, and the whole agitated in a pressure drum for 12 hours or more, and then filtered. Thus the lipoidal substance desired was obtained in the filtrate. The filtrate was then placed in a separating funnel and allowed to separate into layers for several hours. The upper 2 yellow layers of liquid retained. On experimentation it was found that the lower 2 layers contained nothing of value. The retained liquid was then evaporated to vaseline consistency and extracted with acetone, and again filtered. The filtrate was evaporated to dryness and then extracted with distilled water at 37° C. and filtered. Aseptic precautions were taken at every step, and the final solution was placed in sealed flasks in a refrigerator, for standardization.

Determination of Toxicity: Adult male rats were used to determine the toxicity of the solution and varying amounts of the solution were injected to see if there were any ill effects from the possible presence of spermotoxin. It was found that there were no ill effects from the injection of the solution prepared as above.

Measurement of Mormonic Value: Adult bull frogs in hibernation were used for standardization of the solution. These frogs were kept in winter temperature of about 10° C. Their body length was about 8 cm. and they weighed about 50 gm. each. Each frog was tested before injection for the absence of the clasping reflex. A tuberculine syringe was used, and one tenth of a cc. of the solution was injected into the dorsal lymph sac of each frog. The concentrated solution that would just bring the frog into a state of reaction to the clasping reflex was taken as the unit of measurement, and thus the unit of potency.

Conclusions: This is a preliminary report, and although the observations are rather conclusive it is felt that more work needs to be done before it is decided that the method outlined above is the best procedure for the extraction of the male hormone, although it is felt that the clasping reflex is a valuable index for standardization of the potency of the extract.

4460

A Practical Method for the Immunization of Horses by Type Specific Pneumococcic Pleural Exudates.

THEODORE J. CURPHEY AND HERMAN B. BARUCH.
(Introduced by G. B. Wallace.)

From the Simon Baruch Foundation for Research in Pneumonia and the Pathological Laboratories, Bellevue Hospital Medical College.

Following the intratracheal injection into horses of virulent pneumococci of the 3 fixed types in broth cultures, there develops frequently a massive pleural effusion either as a purely local phenomenon or associated with underlying pulmonary consolidation grossly resembling lobar pneumonia, the pleural surfaces of the animals showing all the gross and microscopic evidence of an acute serofibrinous inflammation with the presence of as many as 30 litres of exudate at a time. This exudate besides showing the cellular products of inflammation contains viable pneumococci corresponding to the type injected intratracheally. We have accordingly attempted to immunize horses with this exudate, using ascending doses of the material intravenously. By this method we have been able to produce potent antisera to Type I, and Type II organisms as tested by the mouse protection method. With Type III pleural exudate the resulting antiserum has exhibited same protective value but this has been uniformly low.

The production of type specific antisera by this method presents certain advantages which seem to favor its use in preference to the methods at present in practice. Thus after the exudate has been withdrawn under sterile conditions from the infected animal the material is stored in the ice-box in its native state to be later used in the required dosage for immunization of the serum-producing animals. This naturally lessens the numerous technical steps that are necessary for preparing the immunizing doses of the organism by the present methods.

Besides the technical ease of preparing the antiserum the factor of the length of time taken to produce a potent product is important. From the result of our present observations we are led to believe that this will be materially shortened by this newer method.

Furthermore the degree of antigenic response in our immunized animals is in no way proportional to the number of organisms introduced in that the doses of exudate given intravenously have contained much fewer organisms per cc. than do the immunizing doses of the present methods.

Since the principle of the method described is based on the conception that the antigenic complex that produces immune body response in the course of lobar pneumonia must include certain of the products of tissue cell destruction, we believe that this method has a better biological foundation than the ones in general use at present.

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* We beg to express our sincere gratitude to the Lederle Antitoxin Laboratories for providing us with the facilities of their farm, as well as to Dr. Henry Gordon for his invaluable assistance in the experimental work.

4461

The Ionization of Adsorbed Protein.

HAROLD A. ABRAMSON. (Introduced by W. A. Perlzweig.)

From the Laboratory of Research Medicine, Medical Clinic, the Johns Hopkins University, Baltimore.

The electric mobility of freely dispersed egg albumin and of microscopic quartz particles covered with the same protein have been shown to be fairly similar between pH 3.2 and 5.5.[1] Zeta-potential measurements by the method of streaming potentials have confirmed this relationship.[2] The mobilities of quartz and other kinds of particles covered with egg albumin and with gelatin have since then been studied in the pH range noted in conjunction with the titration curves for these proteins. Under certain conditions, the change in mobility of the protein covered particles has been found to follow the titration curves of these proteins very closely. (Fig. 1.)

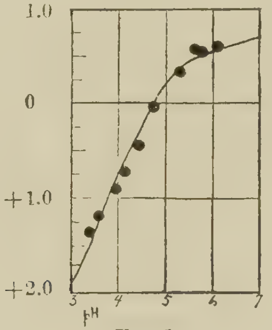

FIG. 1.

The smooth curve is the titration curve for salt-free gelatin taken from Simms.* (A slight change has been made in the ordinate notation.) The dots are values of cataphoretic mobility obtained with gelatin-coated (Licht-filter Agfa) quartz particles suspended in N/150 Na-Acetate at the pH's noted on the abscissa. The protein concentration was 0.2%. It should be noted that the agreement here is only to be had for these conditions. Ordinates are μ/sec./volt./cm. and equivalents of acid per unit quantity of protein.

All of these observations indicate that the process of adsorption of the proteins mentioned does not appreciably change the ionization of the adsorbed protein at the protein-water interface. The free amino and carboxyl groups of the protein are, therefore, not primarily involved in the quartz-protein combination. These facts lend a particular biologic importance to the rôle of protein activity at phase boundaries. In this connection a striking example observed by Michaelis and by Nelson and Griffin[3] demonstrates how an enzyme, invertase, although adsorbed at an inert phase boundary, may still retain its complete activity.

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[1] Abramson, H. A., *J. Am. Chem. Soc.*, 1928, l, 390.

[2] Briggs, D. R., *J. Am. Chem. Soc.*, 1928, l, 2358.

* Simms, H. S., *J. Gen. Physiol.*, 1928, xi, 629.

4462

Differential Cell Count of the Peritoneal Fluid from the Normal Guinea Pig.

LEROY U. GARDNER.

From the Saranac Laboratory for the Study of Tuberculosis, Saranac Lake, N. Y

In carrying out studies on the cellular reactions provoked in the guinea pigs' peritoneal cavity by the injection of tubercle bacilli, differential cell counts were made upon a series of peritoneal fluids previous to the inoculations. This has furnished such a large number of counts upon the normal peritoneal fluid that it has seemed worth while to collect and report the figures, particularly since there is no general uniformity in the results published by other writers.[1, 2, 3, 4, 5, 6] Peritoneal fluids from 237 apparently healthy guinea pigs have been examined, and as no effort was made to select a particular type of animal, this group probably represents a fair average of any laboratory stock. The observations have been carried on over a period of several years during both winter and summer months. Therefore any effects attributable to seasonal or dietary conditions may be assumed to have been comprehended. The majority of the animals were purchased from 4 different sources; a few were raised in the laboratory. They were of both sexes, of many colors, and they varied in age from a few days to several years.

Without previous injection of salt solution or other liquid, samples of peritoneal fluid were withdrawn through sterile capillary pipettes and supravital preparations with neutral red or neutral red-Janus green were made. Differential cell counts were done on 200 living cells after 5 or 10 minutes' incubation in the warm box. At first as many as 1,000 cells were counted, but this was found to be unnecessary as the distribution of cells in the fluid from the normal peritoneum is very uniform if the preparations are properly made. The serosal cells were not tabulated as their occurrence is probably more or less accidental.

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[3] Michaelis, L., *Z. f. physiol. Chemie.*, 1926, clii, 183; *Biochem. Z.*, 1921, cxv, 18. See also Nelson, J. M., and Griffin, E. G., *J. Am. Chem. Soc.*, 1916, xxxviii, 1109.

[1] Szecsi, St., and Ewald, O., *Folia Haematol.*, 1913, xvii, 167.

[2] Lucia, S. P., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 133.

[3] Manwaring and Bronfenbrenner, *J. Exp. Med.*, 1913, xviii, 601.

[4] Kamiya, H., *Beit. z. path. Anat. u. allg. Path.*, 1924, lxxii, 761.

[5] Briscoe, J. C., *Festschr. f. Orth.*, Berlin, 1903, 396.

[6] Cunningham, R. S., *Am. J. Physiol.*, 1922, lix, 1.

TABLE I.

	Grand average 233 animals		Average 150 "Monocyte" animals		Average 83 "Lymphocyte" animals	
		–limits		–limits		–limits
	%		%		%	
Polynuclears	0.12	0-2	0.13	0-2	0.10	0-2
Lymphocytes	17.75	9-75	5.37	0-15	40.10	15-75
Monocytes	67.30	25-95	73.95	25-95	55.70	25-75
Eosinophiles	14.80	0-53	20.60	0-53	4.30	0-25.5

In compiling the results, the counts on 4 animals were discarded because of the presence of acute endemic pneumonia discovered subsequently; they showed from 8.5 to 31.5% of polynuclear leucocytes in the peritoneal fluid. The first column in Table I shows the grand average of the percentages in the remaining 233 animals. In the columns headed "limits" are found the limits of variation for each type of cell and it will be noted that these are extreme. Polynuclear leucocytes are very rare. The monocyte is the most frequently encountered cell and the others are variable.

Further analysis showed that in certain animals the relative number of lymphocytes exceeded that of the monocytes. Because the work on tuberculosis has centered about the lymphocyte-monocyte ratio, the original group was subdivided into one lot in which monocytes predominated, consisting of 150 animals, and another of 83, in which the lymphocyte was the chief cell. A natural point of division seemed to be at 15% of lymphocytes. The differential counts for these sub-groups are tabulated in the second and third columns of the table. It can be demonstrated that for any given animal the monocyte-lymphocyte ratio remains relatively constant unless infection supervenes. For example, 19 of 27 normal animals counted at intervals over a period of 5 months showed less than a 4% variation in these cells. Eosinophiles, on the other hand, are extremely erratic, and in the same animal their relative number changes from day to day.

A complete explanation for the differences in the peritoneal cells is lacking, but certain factors appear to influence the picture. An increase of polynuclears begins to develop within 5 minutes after the injection of any fluid, and of course these cells are already present where acute infection has occurred either in the peritoneum or thorax. The cause of the variable eosinophilia which is apparently not reflected in the cells of the blood might possibly be a local response to a host of protozoan organisms in the nearby intestinal tract. With direct inoculation of tubercle bacilli, the eosinophiles completely disappear from the peritoneal fluid. With regard to monocytes and lymphocytes, the animal showing a majority of monocytes has been considered the "normal" type as 65% of the entire group show this picture. Where lymphocytes are in excess, it has frequently happened that subsequent autopsy has demonstrated the presence of a healing or healed infection at some other point in the body, *e. g.*, otitis media, cholecystitis, organized pneumonia, or enlarged mesenteric lymph nodes possibly associated with a paratyphoid infection. This view is supported by the experience with tuberculous infection, since it has been noted that in a given animal there is always a sudden reversal of the lymphocyte-monocyte ratio which occurs a day or so before the skin becomes sensitive to tuberculin. It is possible that this relationship may also apply to other infections and that a peritoneal lymphocytosis is merely a manifestation of the allergic state. Attempts to explain the differences observed on any other basis have proved unsuccessful. Tabulations of the counts in relation to age, sex, color, diet and meteorological conditions have been unfruitful.

As a result of this analysis it is believed that a differential count of the cells from the "normal" guinea pig is represented by the figures in the second column of Table I.

4463

Effect of Daily Transplants of Anterior Lobe of Pituitary on Reproduction of Frog (Rana pipiens Shreber).

OPAL MARIE WOLF. (Introduced by Frederick L. Hisaw.)

From the Zoological Laboratory, University of Wisconsin.

Smith and Engle[1] first showed that daily transplants of the anterior lobe of the pituitary induced precocious sexual maturity in immature female rats and mice and caused superovulation in these animals as well as those sexually mature. However, Riddle and

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[1] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, xl, 159.

Flemion[2] were not able to induce ovulation in immature pigeons using transplants of this gland. The present investigation was undertaken to determine the effect of daily transplants of the anterior lobe of the pituitary in frogs *(Rana pipiens,* Shreber).

In making the transplant the head of the donor was cut from the body with sharp scissors, the brain exposed, and the anterior lobe was quickly removed from the floor of the cranium and inserted into the lateral or femoral lymph sinus of the recipient. Throughout the experiment the animals were not subjected to temperature changes and were neither fed before nor during the experiment.

In November, 9 out of 11 females receiving transplants either ovulated without amplexus or the uteri were gorged with eggs when the animals were killed. One of the 2 frogs which did not ovulate was killed the fourth day after having received one transplant on 3 successive days. The ovary had apparently been stimulated because the oviducts were highly vascularized. When the second frog died on the eighth day, having received transplants for 4 days, no visible change had occurred in the oviducts.

From the experimental work it has been found that the number of transplants necessary to bring about ovulation varies in individuals. One transplant for 3 or 4 successive days will induce ovulation in most cases, however, 2 animals out of 13 receiving only one transplant ovulated. In no instance has ovulation occurred in the untreated frogs or in the controls which received grafts of brain tissue similar in size to the anterior lobe.

Male frogs also became activated following daily transplants of the anterior lobe of the pituitary for from 2 to 5 successive days. In frogs treated the latter part of November a clasp reflex developed which persisted steadily for over a week but in no case did the males fertilize the eggs of the females. This experiment has been repeated and early in March the males shed sperm over the eggs as they were ovulated, fertilization occurred, and normal development followed.

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[2] Riddle, Oscar, and Flemion, Florence, *Am. J. Physiol.,* 1928, lxxxvii, 110.

4464

Further Observations on the Relationship Between the Metabolism of Galactose and Glucose.*

RALPH C. CORLEY.

From the Department of Bio-chemistry of the School of Medicine, Tulane University, New Orleans.

It is a striking fact that lactose is found in but one place in nature, in the mammary secretion, milk. More information may well be obtained about it and its products of hydrolysis, particularly the galactose moiety. Several investigators have found that tolerance for galactose is less than for a mixture of glucose and galactose. The evidence presented has been the decreased urinary excretion and the less striking change in the blood sugar when glucose accompanied the galactose. One objection to such observations, and a limitation to their interpretation, lies in the absence of knowledge of what changes in galactose concentration of the blood and urine are taking place.

A method for the estimation of galactose in blood and urine has been developed,[1] and applied in an effort to learn more of the relationship between the metabolism of glucose and galactose. Glucose injected simultaneously with galactose has increased the rate of removal of the latter from the blood, but the effect could be explained at least in large measure by the greater urinary excretion resulting.[2] Glucose has been found to have a very marked effect on the amount of galactose appearing in the blood stream when the 2 sugars were fed.[3] However, glucose had an analogous influence on the appearance in the blood xylose, a pentose sugar, and one between which and glucose no metabolic relationship has been shown.[4]

A critical consideration of the data has not entirely ruled out the possibility that glucose has a more intimate connection with the metabolism of galactose, although the results as indicated above can be explained on other bases. Therefore it has seemed of interest to choose different experimental procedures, with the expectation that if the glucose and galactose were to enter the body by different channels, conjugated excretory effects and absorptive interrelationships would be obviated. Rabbits have been used. Experimental details have been described in earlier communications.

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* Assisted by a grant from the David Trautman Schwartz Research Fund.

[1] Corley, R. C., *J. Biol. Chem.*, 1927, lxxiv, 1.

[2] Corley, R. C., *J. Biol. Chem.*, 1927, lxxiv, 19.

[3] Corley, R. C., *J. Biol. Chem.*, 1928, lxxvi, 31.

[4] Corley, R. C., *J. Biol. Chem.*, 1928, lxxvi, 23.

Intravenous injection of glucose simultaneously with galactose, in tartrate nephritis with urinary retention, did not increase the rapidity of removal of galactose from the blood.

Galactose has been fed, and at different intervals, glucose has been injected intravenously. The amount of galactose appearing in the blood has been greatly reduced as compared to control experiments and no increase in the excretion of galactose has been observed. This would seem to be fairly unobjectionable evidence of an actual metabolic influence of glucose on the disposal of galactose, since it is difficult to conceive that parenterally introduced glucose could affect the rate of absorption of another sugar from the gut, although this contingency is not to be disregarded in the absence of directly pertinent evidence.

Galactose has been injected intravenously at different stages of the absorption of glucose. No striking influence on the rate of removal of the former sugar from the blood has been observed. It is of interest to record, however, that the rate of disposal appeared appreciably faster when injection was made 2 hours after as compared to 1 and 3 hours after the feeding of glucose. A possible explanation may be that there is an evocation of insulin that exerts a maximum influence 2 hours after feeding glucose. Further investigations along these lines might yield valuable information in regard to the action of insulin in carbohydrate metabolism.

Subcutaneously injected insulin has been found to increase the ease of disposal of orally and intravenously administered galactose. Confirming an earlier report,[1] simultaneously injected insulin increased the rate of disposal of galactose from the blood.

This influence of insulin on the metabolism of galactose furnishes a basis for several interesting speculations. Any interrelationship between the metabolism of glucose and galactose might be ascribed to a mobilization of insulin, or the increased ease of disposal of galactose might be tied up with the simultaneous oxidation of glucose, in which insulin plays such an important rôle. After a subcutaneous injection of a mixture of glucose and insulin, circulating galactose disappeared more rapidly. Furthermore the simultaneous intravenous injection with galactose of glucose and insulin or of levulose and insulin increased the rate of disposal of galactose from the blood. These influences may of course be dependent on a conjugated oxidation with glucose, or may merely mean that with the quantities employed, there was an excess of insulin which exerted its action directly.

While this question has innumerable ramifications, and many of the observations can be explained on the basis of other considerations, there still remains the impression that in some way the metabolism of galactose is intimately related to that of glucose. Further studies along these lines are in progress.

4465

Purification of Diphtheria and Other Bacterial Toxins by Adsorption.

PAUL GROSS.* (Introduced by E. E. Ecker.)

From the Department of Pathology, School of Medicine, Western Reserve University, Cleveland, Ohio.

Current methods of purification of diphtheria toxin include precipitation by acidification or by the addition of salts, and dialysis or ultrafiltration. Glenny and Walpole[1] by a combination of ultra filtration and acidification have succeeded in attaining a relatively high degree of purification. The method of adsorption, however, has not received sufficient attention. Various adsorbants such as calcium phosphate, aluminum chloride and zinc salts have been used with indifferent results, due either to poor adsorption or to destruction of the toxin. That adsorption can be almost complete has been demonstrated by Coplans,[2] who showed that finely divided asbestos removes 99.9% of diphtheria toxin from its broth.

Recently Fuchs[3] succeeded in adsorbing prothrombin to magnesium hydroxide. This fact suggested the use of magnesium hydroxide for the isolation of diphtheria toxin. It was found that when magnesium hydroxide (in colloidal suspension) was added to diphtheria broth, adsorption of the toxin occurred. The supernatant liquid retained less than 4% of its original toxicity. Following repeated washings with distilled water and subsequent treatment of the precipitate with CO_2 or ammonium phosphate the toxin was recovered. Dialysis removed traces of magnesium and ammonium salts. Injection of the adsorbed toxin into guinea pigs produced death with hemorrhagic adrenals and a gelatinous peritoneal exudate. Control animals receiving 10 times this amount of adsorbed toxin mixed with antitoxin survived. Titration of the adsorbed toxin by the usual method indicated a degree of adsorption in excess of 94%. The M.L.D. of the original toxin was 0.0035 cc. while that of the adsorbed toxin was less than 0.0037 cc.

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* Crile Research Fellow in Pathology.

[1] Glenny, A. T., and Walpole, G. S., *Biochem. J.*, 1915, ix, 298.

[2] Coplans, M., *J. Path. and Bact.*, 1914, viii, 581.

[3] Fuchs, H., *Z. f. Immunitätsf.*, 1928, lviii, 14.

Nitrogen determinations (by micro-Kjeldahl) indicated that the nitrogen loss sustained during the process of purification was in excess of 90%.

Tetanospasmin and staphylolysin were similarly adsorbed to and eluted from the magnesium precipitate. Tetanolysin, however, was destroyed.

Conclusions: (1) Magnesium hydroxide in colloidal suspension adsorbs diphtheria toxin which can be eluted without destruction of the toxin. (2) The adsorption is approximately 94% complete. (3) The nitrogen loss per M.L.D. sustained during the process is in excess of 90%. (4) The method has been used with success in the adsorption of tetanospasmin and staphylolysin but not with tetanolysin and indicates possibilities for the purification of other bacterial products.

Thanks are due Prof. E. E. Ecker for many helpful suggestions and criticisms.

4466

Further Observations Concerning the Effect of Posture on the Velocity of Blood Flow in Man.*

WILLARD OWEN THOMPSON† AND JOSEPH M. ALPER.

From the Metabolism Laboratory of the Massachusetts General Hospital, Boston.

We have previously shown that a much longer time is required for blood to move from an arm vein to a foot vein or the reverse, when an individual is in the standing still position than when he is in the recumbent position.[1, 2, 3] This observation was in harmony with the work of several observers[4, 5, 6, 7, 8] who have shown that, in

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* Aided in part by a grant from The Proctor Fund of the Harvard Medical School, for the Study of Chronic Diseases.

† Fellow of the National Research Council, Research Fellow in Medicine, Harvard Medical School and Massachusetts General Hospital.

[1] Thompson, W. O., Alper, J. M., and Thompson, P. K., *J. Clin. Invest.*, 1928, v, 605.

[2] Thompson, W. O., Thompson, P. K., and Dailey, M. E., *Proc. Nat. Acad. Sci.*, 1928, xiv, 94.

[3] Thompson, W. O., Thompson, P. K., and Dailey, M. E., *J. Clin. Invest.*, 1928, v, 573.

[4] Piorry, P. A., *Arch. Gén. de Méd.*, 1826, xii, 527.

the standing still position, blood circulates with difficulty and collects in dependent portions of the body owing to the effect of gravity. In view of these findings, it seemed to be a reasonable hypothesis that the slowing of the circulation would take place below, and not at the level of the heart. We have now investigated this question experimentally by measuring the arm-to-arm and foot-to-arm circulation times in the recumbent and in the standing still positions in several normal subjects, using an improvement‡ of a dye method previously described.[1] Numerous observations on 6 subjects have

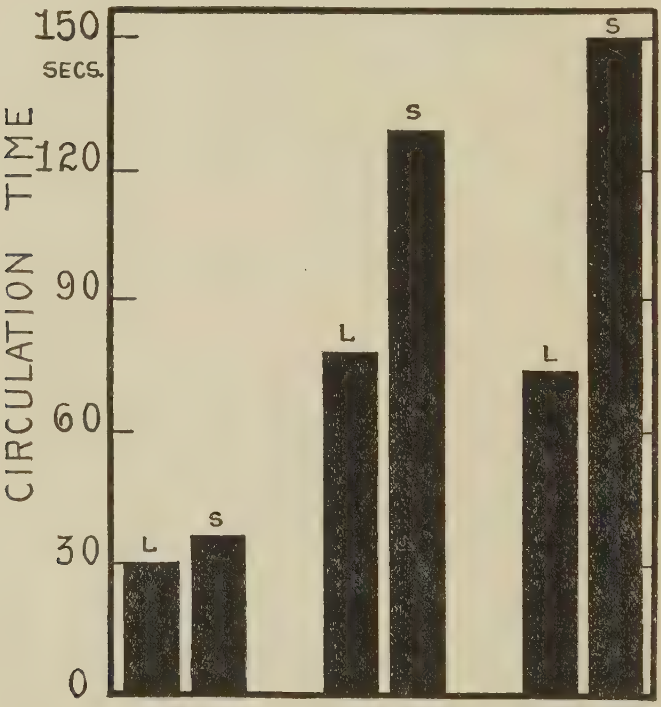

FIG. 1.

A comparison of arm-to-arm and foot-to-arm circulation times in the recumbent (L) and in the standing still (S) positions. Subject, J. G.

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[5] Salathé, A., *Trav. du lab. de M. Marey*, 1877, 251.

[6] Hill, L. J., *J. Physiol.*, 1895, xviii, 15.

[7] Field, H., Jr., and Bock, A. V., *J. Clin. Invest.*, 1925, ii, 67.

[8] Turner, A., *Am. J. Physiol.*, 1927, lxxx, 601.

‡ The chief improvements were, changing the tubes in which blood was collected to determine the presence of dye at more frequent intervals (5 and 10 seconds instead of 15), and using an anticoagulant (isotonic sodium oxalate) which made detection of the dye easier.

shown that as a rule the arm-to-arm circulation time is about the same in both positions of the body; whereas the foot-to-arm circulation time is much longer in the standing still than in the recumbent position. Data on a typical case are recorded in Fig. 1.

Our results are consistent with the theory that in the standing still position, the circulation is slowed only where it is impeded by gravity, *i. e.,* in dependent portions of the body.

4467

Experimental Herpetic Encephalitis in the Guinea Pig Produced Through the Respiratory Tract.

EARL B. MC KINLEY.

From the School of Tropical Medicine of the University of Porto Rico, under the auspices of Columbia University, San Juan, Porto Rico.

In a previous communication[1] we described experimental *herpetic encephalitis* produced by feeding herpes virus to guinea pigs. The brains from such animals, dying after feeding of herpes virus, when injected into rabbits by the intraspinous route produced typical *herpetic encephalitis* and the disease could be passed in series from rabbit to rabbit. Control cultures of the guinea pig brains were without growth. It was noted that the herpes virus was somewhat attenuated following passage through guinea pigs by this method but its virulence rapidly returned by rabbit passage. It was further observed that only about 50% of the guinea pigs so treated with herpes virus died, the others remaining apparently unaffected. The "Beckley" strain of herpes (Flexner) was used in these experiments.

The apparent passage of the herpes virus from the intestine of the guinea pig to the brain, as demonstrated by subsequent passage through rabbits, has led us to consider the possibility of infecting guinea pigs by dropping herpetic brain emulsion into the nostrils of these animals.* In these experiments we have employed the Le Fèvre herpes strain which has been fully described in another publication.[2]

An emulsion of herpetic (Le Fèvre) rabbit brain was prepared by emulsifying one gm. of infected brain in 5 cc. of physiologic salt solution. In the first experiment 3 guinea pigs were given 10 drops of this emulsion in each nostril. Three control guinea pigs received a similar quantity of an emulsion prepared with normal rabbit brain. Seven days after treatment one of the guinea pigs became ill and on the eighth day was in coma and had repeated convulsions. The animal remained in this state for 30 hours and was sacrificed late on the ninth day. The brain was removed and placed in 50% glycerol. Three days later an emulsion was prepared (1 gm. of the suspected guinea pig brain emulsified in 5 cc. of saline) and 0.5 cc. of this emulsion was injected intraspinously into a normal rabbit. The other 2 guinea pigs which had received infected rabbit brain remained well as did the 3 control guinea pigs which had received in each nostril 10 drops of a normal rabbit brain emulsion. The rabbit so injected with the suspected guinea pig brain died with typical herpetic encephalitis 7 days following inoculation. The virus was then passed serially through 6 rabbits and in each case the rabbit so inoculated died with typical symptoms of herpetic encephalitis within 5 or 6 days. In each case control cultures made from the rabbit brains were without growth. Emulsions of normal guinea pig brain injected intraspinously into rabbits proved to be innocuous.

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[1] McKinley, E. B., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 21.

* Somewhat similar and likewise positive results on nasal infection in rabbits by herpes virus have been reported by Levaditi and Harvier, Da Fano, Remlinger and Bailly, and Flexner and Amoss.

[2] McKinley, E. B., and Holden, M., *J. Infec. Dis.*, 1926, xxxix, 441.

In a second series we confirmed these results upon a larger scale. Of 12 guinea pigs receiving herpes emulsion intranasally only 2 died. Typical encephalitis was produced in rabbits with the brains from these 2 animals. In this experiment 6 control guinea pigs were used, each receiving 10 drops of normal rabbit brain emulsion intranasally. All remained unaffected. The remaining 10 guinea pigs receiving infected material remained perfectly well after nearly 2 months following treatment.

These experiments indicate that, in certain cases, the virus of herpes may reach the brain of guinea pigs following deposition of the virus in the nares of such animals. That only a few animals so treated become infected is significant. There is without doubt a certain individual resistance to this virus when the animal is infected in this manner. Dmitrieff[3] noted that there is a tendency for herpes strains to be reduced in virulence when passed through guinea pigs by intracerebral inoculation. Similar observations were made by Rose and Walthard.[4] Flexner[5] states that in guinea pigs the effects of the inoculations vary with the strength or degree of virulence of the virus. He believes that if Dmitrieff and Rose and

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[3] Dmitrieff, S., *Z. Hyg. u. Infektionskrankh.*, 1926, cvi, 547.

[4] Rose, G., and Walthard, B., *Z. Hyg. u. Infektionskrankh.*, 1925-26, cv, 645.

[5] Flexner, S., *J. Exp. Med.*, 1928, xlvii, 23.

Walthard had employed strongly virulent strains their results could not but have been very different. On the whole we feel that there is good evidence in our experiments that even the highly virulent strains, such as the Beckley and Le Fèvre strains, become somewhat attenuated by passage through guinea pigs by the feeding and inhalation methods of infection which we have employed. However destruction of the virus does not take place and the lost virulence of the virus is quickly regained by passage through rabbits. Our experiments would however, we believe, lend support to Flexner's concept that the guinea pig serves to separate weak from strong strains of herpes. Olitsky and Long[6] have shown that a weak strain, such as the Levaditi C strain, may fail to produce characteristic encephalitis in guinea pigs though the virus may be present in sufficient quantity to permit its detection. These authors also observed that the Levaditi virus failed to elicit signs of infection in guinea pigs following repeated intraperitoneal injections. Judging from our observations on the infrequency of producing infection in guinea pigs by feeding and intranasal inoculations with highly virulent strains we would suspect that infection would be impossible with weaker strains by these methods.

Practically nothing is known concerning the portal of entry of the true virus of epidemic encephalitis in man, and while there is little evidence to support the view that epidemic encephalitis is caused by the herpes virus, the possibility of infection by such a virus through the respiratory tract may prove to be of importance from the epidemiological point of view. The apparent resistance of certain individuals when inoculated in this manner may also have its counterpart in human beings. Since the virus of herpes has so rarely been found in the brains of human cases dying of encephalitis it is suggested that the virus be sought for elsewhere. If the natural infection may take place through the respiratory tract, and if, through some mechanism, the true agent may lose its virulence in the central nervous system and thus escape detection, one might consider the possibility of finding it in some other locality, such as the respiratory tract.

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[6] Olitsky, P. K., and Long, P. H., *J. Exp. Med.*, 1928, xlviii, 379.

4468

The Isoelectric Point of Gelatin in Relation to its "Minimum Physical Properties."

J. M. JOHLIN. (Introduced by G. E. Cullen.)

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Most investigators are of the opinion that the physical properties of gelatin (viscosity, conductivity, osmotic pressure) are at a minimum at its isoelectric point and that its opacity is a maximum at this point. This erroneous conception is partly due to the notion that ash-free gelatin is necessarily isoelectric. Gelatin, whose high degree of purity is indicated by an exceptionally low conductivity when it is free from diffusible electrolytes is not isoelectric. Cataphoresis experiments show that purified gelatin derived from lime conditioned stock migrates to the anode in an electrical field, while gelatin from acid conditioned stock migrates toward the cathode. The former must therefore be a weak acid while the latter is a weak base. The physical properties of gelatin are at a minimum in pure gelatin and not in isoelectric gelatin, which is a salt of gelatin and more highly ionized than pure gelatin.

Pure gelatin from any source should have the formula $HONH_3\,X\,COOH$ (where X represents the main gelatin radical and the other groups are representative of its acid and basic groups) and should accordingly furnish the ions $HO^-\,NH_3^+\,X\,COOH^+$. In the case of gelatin, which is a weak acid, the number of ionized groups furnishing H^+ would be greater and in the case of gelatin which is a weak base the number of groups furnishing OH^- ions would be greater, so that the 2 types of ionized gelatin may be represented by $(HONH_3\,X\,COO)^-\,H^+$ and $HO^-(NH_3\,X\,COOH)^-$ respectively.

It can be seen readily that the addition of an acid (HCl) to the type of gelatin $(HONH_3\,X\,COO)^-\,H^+$ will partly suppress its ionization and react with some of the OH groups of the molecule to form a chloride of gelatin $ClNH_3\,X\,COOH$ which would dissociate to furnish the more highly ionized $Cl^-\,NH_3^+\,X\,COO^-\,H^+$, just as the addition of HCl to NH_4OH forms the more highly ionized NH_4Cl.

If the proper amount of acid is added to pure gelatin, a point must be reached where the $\overline{Cl}$ ions equal the H^+ ions in number. (More correctly the $\overline{Cl} + \overline{OH}$ ions equal the H^+ ions.) At that point the gelatin radicle would have equal positive and negative charges and be, according to the correct interpretation of the term, isoelectric. Such an isoelectric, ionized salt of gelatin must necessarily have an osmotic pressure, viscosity and conductivity which is higher than that of pure gelatin.

Pure gelatin from acid conditioned stock furnishes the ions $HO^- NH_3^+ X COOH^+$. The addition of a base (NaOH) would suppress this ionization and at the same time form a salt $HO NH_3 X COO Na$ furnishing the ions $HO^- NH_3^+ X COO^- Na^+$. If the proper amount of base is added, a point must be reached where the positive and negative charges on the gelatin radicle are equal, and it is isoelectric.

It would seem evident then that the isoelectric point of gelatin is still an unknown quantity, and there are, at present, no methods for its exact determination. Any attempt to study the migration of gelatin in a gelatin solution would result in a destruction of the balance of diffusible ionic groups necessary to maintain the isoelectric point. Any attempt to study the migration of gel particles of gelatin by direct observation are open to the objection that the electric charge on such particles is partly due to selective adsorption and not entirely to ionization.

The purified gelatin which Loeb[1] prepared at a pH of 4.7 for his classic experiments may have been somewhat nearly isoelectric gelatin but its physical properties were not at a minimum. Dhere's criticism[2] that this gelatin was not free from ionic impurities is justified. Northrup and Kunitz,[3] who have recently criticized Dhere's point of view have apparently misinterpreted Dhere's criticism of Loeb's gelatin. In another part of Dhere's paper he states clearly the difference between isoelectric gelatin which is a compound containing "foreign ions", and "free gelatin" which is gelatin free from diffusible ionic impurities, although he does not state clearly the exact nature of isoelectric gelatin. His contention is that Loeb's gelatin was not free from diffusible ions, and that gelatin free of such ionic impurities is not isoelectric because it is negatively charged. In other words Loeb's gelatin could not be isoelectric and free of diffusible ionic impurities at the same time. It must not be overlooked that isoelectric gelatin is in part a salt of gelatin and is more highly ionized than pure gelatin but that the number of positive charges contained in the gelatin radicle are equal to the number of negative charges which it contains. The assumption sometimes made that isoelectric gelatin is not ionized at all is erroneous.

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[1] Loeb, J., Proteins and Theory of Colloidal Behavior, 1922, McGraw-Hill.

[2] Dhéré, C., *Kolloid Zeit.*, 1927, xli, 315.

[3] Northrop, J., and Kunitz, M., *J. Gen. Physiol.*, 1928, xi, 478.

Northrup and Kunitz definitely appreciate the fact that isoelectric gelatin must have a diffusible anion present (and it should be added "or cation" if the gelatin is, by any chance, of acid conditioned stock). It would seem, however, that they, as well as Hitchcock,[4] who uses their method of purification, render their gelatin free of a part of the acetic acid necessary to maintain it at its isoelectric point when they precipitate it and wash it with alcohol and ether. At any rate, the pH for the new isoelectric point which Hitchcock recently gave for ash free gelatin is more nearly that of pure gelatin, free from diffusible ionic impurities, and the conductivity of this gelatin is also that of Cooper's purified, electrolyte-free gelatin which was recently purified electrolytically by the method described by the writer some years ago.

This new concept of isoelectric gelatin and its relation to the physical properties of gelatin in no way diminishes the validity or importance of Loeb's work or of that of many other investigators who have helped to develop the concept that proteins are amphoteric electrolytes which react with acids and bases in a perfectly normal manner. It helps rather to emphasize this point of view and to clarify the confusion which now exists because of the variable pH of different proteins and of different concentrations of proteins. This critical point of hydrogen ion concentration, where proteins have these "minimum physical properties," previously referred to as the isoelectric point, now becomes the point where the protein in its purest form exists as an acid or a base, and has a pH which is a function of the ionization constants of the protein in question.

Data and references in support of some of the contentions which have been made will be given in detail later.

4469

Effect of Feeding Whale Oil on the Depot Fat of the White Rat.

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From the Department of Physiological Chemistry, College of Medicine, Ohio State University, Columbus, Ohio.

Forty-three female white rats of average weight 154 gm. were fed for 6 weeks on a diet of 62.5% whole wheat flour, 15% casein, 15% skim milk powder, 1.5% precipitated chalk, 1% NaCl, 5%

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[4] Hitchcock, David, *J. Gen. Physiol.*, 1929, xii, 495.

cotton seed oil and 0.3% cod liver oil. Following this control diet, the animals were changed to one containing 10% whale oil and 90% control mixture for 2 weeks, and from then on to a diet of 20% whale oil and 80% control. After 10 weeks of whale oil feeding the control diet was resumed. At intervals during the experiment groups of 6 rats were killed and the depot fat was removed by dissection and rendered. The results of the analysis of the specimens of fat are given in Table I.

TABLE I.

Time Weeks	Fat				Fatty Acids	
	Iodine No.	Sapon. No.	Ref. Index	Polybromide No.	Polybromide No.	% Br. in Bromides
			20°			
0*	86.06	200.8	1.4677	0.	— —	— —
2	88.46	197.2	1.4694	3.53	— —	— —
4	96.73	193.7	1.4702	6.70	— —	— —
6	97.26	193.1	1.4702	13.08	14.08	67.53
8	97.88	193.1	1.4703	14.10	14.98	67.61
10	97.90	193.2	1.4703	14.15	15.12	67.63
13†	94.27	195.2	1.4700	10.03	10.82	67.58
Whale oil	127.32	189.1	——	24.20	25.24	67.58

*After 6 weeks on control diet.
†After resumption of control diet for 3 weeks.

From the above data it is concluded that equilibrium between the food fat and the depot fat is established within 4 to 6 weeks on a given diet. The characteristic highly unsaturated fatty acids of whale oil, including acids of the C_{18}, C_{20} and C_{22} series with 4, 5 and 6 double bonds, are apparently deposited as such since the bromine content of the polybromides is practically the same as that of the original whale oil. (Chemically whale oil is classed with the fish oils.) A maximum polybromide number of 15.12 containing 67.63% bromine indicates a storage of at least 4.9% of unsaturated acids on a 20% whale oil diet. On the other hand, these acids give a polybromide number only about 1/3 of the theoretical,[1] from which a storage of nearly 15% may be calculated. Resumption of the control diet for 3 weeks still showed a considerable quantity of highly unsaturated acids to be present. When once stored, they are apparently removed only slowly.

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[1] Brown, J. B., and Beal, G. D., *J. Am. Chem. Soc.*, 1923, xlv, 1289.

4470

The Dilator Effect of Histamine on the Cerebral Vessels in Man.

SOMA WEISS, WILLIAM G. LENNOX AND GEORGE P. ROBB.

From the Thorndike Memorial Laboratory, Boston City Hospital, and the Department of Medicine, Harvard Medical School.

Direct evidence as to whether or not the cerebral vessels in man possess vasomotor play is still lacking.

The following observations on patients indicate that the arterioles, capillaries, and venules of the human brain respond with prompt dilatation to histamine.

Following the intravenous administration of an average dose of 0.07 mg. (0.7 cc. of a solution of 1:10,000) of histamine phosphate the spinal fluid pressure showed a marked rise. The onset of this rise occurred about simultaneously with the circulation time of the injected histamine. During and slightly after the rise of the spinal fluid pressure the excursions of the oscillatory pulsation of the spinal fluid were greatly increased, although there was no demonstrable rise in the simultaneously registered arterial or venous blood pressure. Coincident with these objective changes there was a sensation of throbbing and pressure in the head.

A small amount of histamine phosphate administered intravenously, when the hemisphere was exposed for the purpose of removal of tumor, caused a prompt bulging of the brain, a distinctly increased cerebral pulsation, and a visible bright flush of the brain surface. These changes, just as other bodily responses following the single intravenous dose, lasted but 0.5 to 3 minutes. Forbes, Wolf and Cobb have observed a similar behaviour of the small pial vessels of the cat.

Adult subjects tolerated well the continuous intravenous administration of 0.05 to 0.1 mg. of histamine phosphate per minute of a solution of 1:10,000. The oxygen and carbon dioxide content of the arterial blood flowing to, and the venous blood returning from the brain was measured. It was observed that while the arterial blood during the administration of histamine showed an average increase of 8% in the oxygen content, because of increased concentration of the blood; the oxygen content of the blood obtained from the internal jugular vein showed an average rise of 20% over the value found before the injection of histamine. During identical experimental conditions the average increase in the oxygen content of the blood obtained from the external jugular vein was 12%, and that from the basilic vein 24%. The average decrease of the carbon dioxide content of the blood obtained from the internal jugular vein was 9%.

Among the 9 subjects whose blood from the internal jugular vein was studied, 2 failed to show a rise in the oxygen content during the administration of histamine. As rise in the spinal fluid pressure occurs with regularity following histamine injection, one has to conclude that histamine in these individuals produced only a dilatation of the venules. Further evidence that histamine may induce dilatation of the veins directly is revealed through observations of the small veins of the conjunctiva which respond with a dilatation following continuous administration of histamine. Diffuse dilatation of the subpapillary venous plexuses of the skin also developed in certain persons during the administration of histamine, without simultaneous arteriolar dilatation.

One part of epinephrin chloride antagonized the effect of 10 parts of histamine phosphate on the minute vessels of the skin. Epinephrin chloride in the same ratio also abolished the changes induced by histamine in the cerebral vessels.

The evidence thus presented indicates that histamine produces marked vasomotor response in the minute vessels of the brain. Comparative quantitative observations demonstrate that the sensitivity of the cerebral vessels to the minimal effect of histamine is marked, and the sensitivity is at least as great as that of the facial vessels, which are the most sensitive of the cutaneous vessels. The sensitivity of the cerebral vessels to histamine and epinephrin suggests that these chemical substances through their local action may play a rôle in the physiologic and pathologic regulation of the cerebral circulation in man. Whether or not, in addition to the local *chemical vasomotor regulation*, direct central nervous vasomotor regulation of the cerebral vessels is active in man, is still an open problem.

4471

Thirteen Thousand Generations of Paramecium.

LORANDE LOSS WOODRUFF.

From the Osborn Zoological Laboratory, Yale University.

As a matter of record it seems well to summarize the data and give the present status of the long-continued culture of *Paramecium aurelia* at Yale University.

Can unicellular animals reproduce indefinitely without recourse to fertilization? This was the question to which an answer was sought when this experiment was begun 22 years ago. On May 1st, 1907, a pedigree culture of *Paramecium aurelia* was started with a "wild" individual and has been maintained by the isolation of certain of its progeny practically every day, with the exception noted below. The number of divisions has been recorded at each isolation and has afforded a continuous series of data for the study of the reproductive activity of the culture. Throughout the experiment some of the animals discarded from the lines at the daily isolations have been fixed, stained, and mounted as permanent preparations for the study of the cytological changes during the life history. The culture medium for the first 8 months of the work consisted of infusions of hay and fresh grass, but since February, 1908, various materials collected from ponds, swamps, etc., have been employed. The infusions have invariably been thoroughly boiled to prevent the contamination of the pure culture with foreign strains of Paramecium. The possibility of conjugation occurring in the culture has been precluded by the almost daily isolation of the products of division. In short, the animals of the culture are all direct lineal descendants, without fertilization, of the single animal isolated in 1907.

In this manner the pedigree culture was carried for 8 years, during which 5071 generations were attained, and then (May 1, 1915), the experiment was considered formally closed with the statement that the organisms of the present generation are in as normal morphological and physiological condition as the original individual isolated to initiate the culture.

However, with this conclusion secure, I was reluctant to discard the race and have kept it under control, but without such exacting daily observation and recording of the daily division rate. Accordingly, it has been carried in this manner up to the present time (May, 1929). But from time to time 30-day tests have been made of the vitality of the race under the former rigid culture conditions, and in each case the same general average division rate has been revealed as during the first 8 years of its life. On the basis of such tests it is fair to make the exceedingly conservative estimate of 600 generations attained each year since May 1, 1915. This would give, in round numbers, 13,500 generations attained by the culture during the 22 years of its life to date since May 1, 1907. The vitality of the culture is further attested by the fact that it is continuously affording animals for various other experimental studies on Paramecium at Yale and elsewhere.[1]

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[1] Woodruff, L. L., *Am. Naturalist*, 1925, lix.

4472

Some Further Observations on Sodium Iso-Amyl-Ethyl-Barbiturate as a Laboratory Anesthetic.

CHARLES C. LIEB AND MICHAEL G. MULINOS.

From the Department of Pharmacology, College of Physicians and Surgeons, Columbia University.

The insoluble iso-amyl-ethyl-barbituric acid (amytal) may be dissolved by neutralization with 15% NaOH and gentle heat.[1] Subsequent clouding of the solution may be cleared by filtration, or by more NaOH. The experiments reported below show that such treatment does not decrease the anesthetic power, nor increase the toxicity, of the drug.

Anesthesia has been produced in human beings by the intravenous injection of solutions of the anhydrous sodium amytal.[2] It is stated in the report cited[2] that the solutions made by neutralization are more toxic and less effective than those made from the pure sodium amytal. This anesthetic is used in the laboratory to produce anesthesia for the study of problems involving carbohydrate metabolism. Does the increase in toxicity reported affect in any way the mobilization of glucose?

Solutions of sodium amytal made by neutralization of the acid, and from the anhydrous powder of pure sodium amytal* were injected intravenously into cats, rabbits and dogs. Previous to the injection the animals were anesthetized with ether, and the blood pressure recorded. The right vagus was stimulated just before and after each injection. The drugs were diluted so that 1 cc. of the solution contained 25 mg. The sodium amytal solution by neutralization was diluted from the stock 10% solution just before the injection; that from the pure sodium amytal was made a few minutes before use. Samples of such solutions were kept for varying periods, and compared with the 2 above as to toxicity, effectiveness, and stability. No difference could be found between any of the samples injected. No increase in toxicity was evident after the solutions had stood from 1 to 60 days. No experimental evidence could be adduced that these solutions are influenced by exposure to air.

It was found that intravenously injected sodium amytal solutions impaired the effects of vagus stimulation on the heart. This is very well illustrated on the accompanying chart. Here is also shown the toxic effect of a freshly prepared solution of sodium amytal from the pure anhydrous powder.

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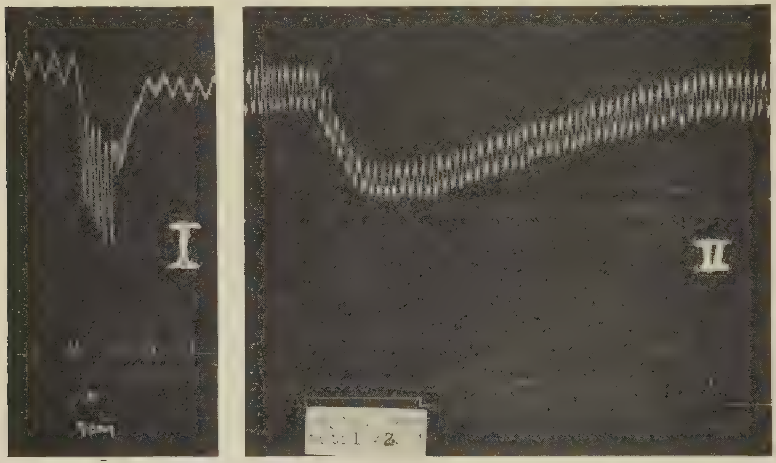
[1] Mulinos, M. G., *J. Pharm. Exp. Ther.*, 1928, xxxiv, 425.

[2] Zerfas, L. G., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 399.

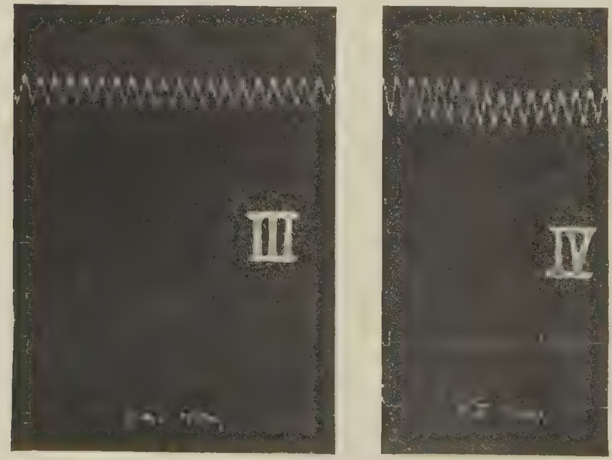
* Obtained through the kindness of Eli Lilly & Company.

CHART.

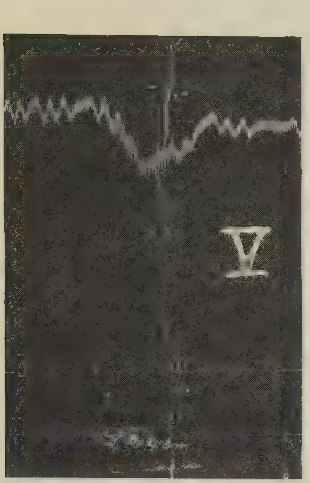
Cat, F., weight 3.4 kg. Ether anesthesia.
B.P. through left carotid artery. Injections into right jugular vein.
V.D. = Right vagus, cut; inductorium at 4 cm. Time = 5 sec.

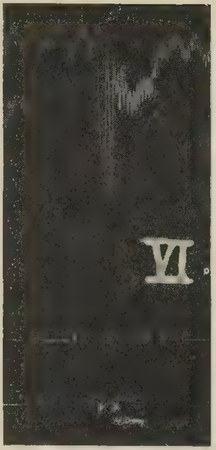


I. Vagus stimulated before any amytal.
II. Effect of 20 mg. sodium-iso-amyl-ethyl-barbiturate (Lilly) intravenously, to total 37.5 mg. Fluid injected = 0.8 cc.



III. Vagus stimulation 5 min. after a total of 50 mg. per kg. of amytal sodium had been given.
IV. Vagus stimulation 45 min. after the drug.





V. Vagus stimulation 1.5 hrs. after the drug.
VI. Vagus stimulation 2.1 hrs. after the drug.

Summary: (1) Solutions of sodium amytal injected intravenously depress the circulation. (2) Such solutions cause prolonged but temporary paralysis of the vagus to the heart. (3) These solutions are stable, and do not show any increase in toxicity within 2 months.

4473

A Photographic Method of Recording Plethysmograms.

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(Introduced by Charles Sheard.)

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Measuring the change in volume of an organ is done ordinarily by placing the organ under investigation in a plethysmograph and computing the change in volume that occurs in the air space between the organ and the interior wall of the plethysmograph. The change in volume manifests itself by a change in pressure when the plethysmograph is a portion of a closed system so that if, for example, the organ expands, the volume of the system decreases and the pressure increases. Hence by noting the change in pressure it is possible to follow the corresponding change in volume of the organ.

A record of the change in pressure may be made by one of several methods. A Marey tambour or a piston recorder may be used in conjunction with a kymograph and a tracing made on smoked paper. Besides the possibility of leakage in the tambour or piston, there is always the difficulty of maintaining the proper adjustment of the stylus against the smoked paper. On the other hand, a U-tube containing a liquid may be used satisfactorily as a manometer. Hargis,[3] in his measurements on the changes in volume of the spleen, used a water manometer and followed manually the rise and fall of the meniscus, making a record on smoked paper. Although the latter method of obtaining changes of pressure is satisfactory, the mechanism for recording is only grossly accurate, requires the constant attention of the recorder, and is not capable of registering very rapid fluctuations such as may occur at every heart beat.

We have devised a simple method of recording accurately the fluctuations in the position of the meniscus of a water manometer. If a piece of ordinary glass tube, with a bore of approximately 3 mm., is partly filled with water and held at right angles to the rays

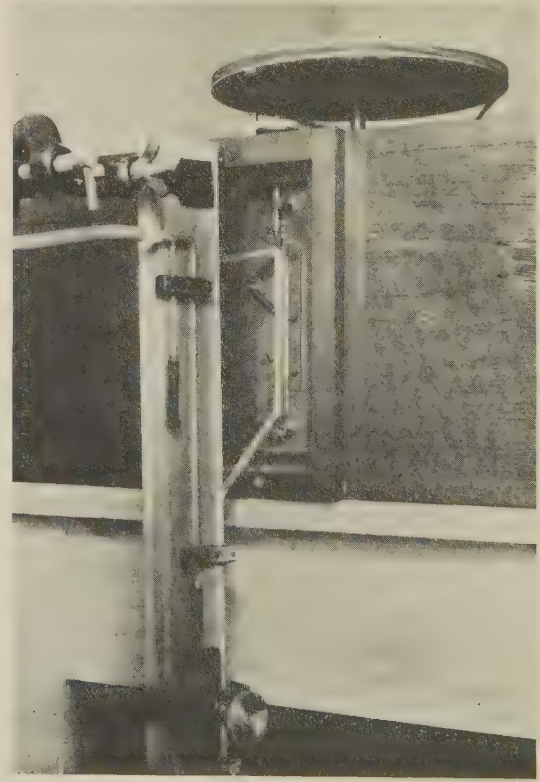

FIG. 1.
The ensemble for the photographic method of recording plethysmograms.

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[3] Hargis, E. H., *J. Lab. and Clin. Med.*, 1926, xi, 664.

of light coming from a powerful Mazda lamp a few meters distant, it will be noticed, on placing a piece of paper about 3 mm. behind the glass tube, that the column of water focuses the light sharply on the paper whereas the glass tube alone does not. In other words, the portion of the tube containing water functions as a cylindrical lens and as the water in the tube moves up and down the concentrated beam of light follows accordingly. If, then, the customary cylindrical lens of a recording camera of an electrocardiograph is replaced with one arm of a U-tube partly filled with water, it is possible to photograph changes of pressure by following the elevations or depressions of the meniscus, as evidenced by change in length of the cylindrical lens.

A photograph of the recording apparatus is shown in Fig. 1. A Cambridge electrocardiographic camera using paper 8 cm. (about 3.25 inches) in width is turned on its side and the cylindrical lens in front of the slit removed. The glass tube is shown in front of the slit, whereas in actual use it is pressed firmly against the slit. The height of the meniscus is controlled by pressure on the rubber tube at the bottom of the manometer. Usually the meniscus is kept as high as possible so that a considerable portion of the paper is exposed. A speed of 5 mm. each second has been found satisfactory although this can be varied by changing the pulley ratio between the camera and the Leeds and Northrup constant speed motor which is used for driving the roll of paper in the camera proper. A 250 watt Mazda projection lamp is used as a source of light and, at a distance of about 4 meters, has been found to produce sufficient exposure of the paper.

This apparatus is adapted readily to recording the motion of the shadow of a pointer which is indicative of the motion of the pointer itself. For instance, a Marey tambour may be placed a few centimeters in front of the camera so that the shadow of the pointer falls on that portion of the glass tube which acts as a cylindrical lens. The shadow of a pointer 1 or 2 mm. wide leaves an excellent tracing on the developed photographic record. Besides one or two tambours, a signal magnet and a time marker may be used ordinarily in conjunction with the water manometer, and records of the movements made by each of these may be obtained on paper only 8 cm. wide.

This apparatus was used in the experimentation on the effects of acoustic stimulation on the volume of the spleen and kidney which are presented in other papers.[1, 2]

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[1] Corbeille, Catherine, and Baldes, E. J., *Am. J. Physiol.*, 1929.

[2] Corbeille, Catherine, and Baldes, E. J., *Am. J. Physiol.*, 1929.

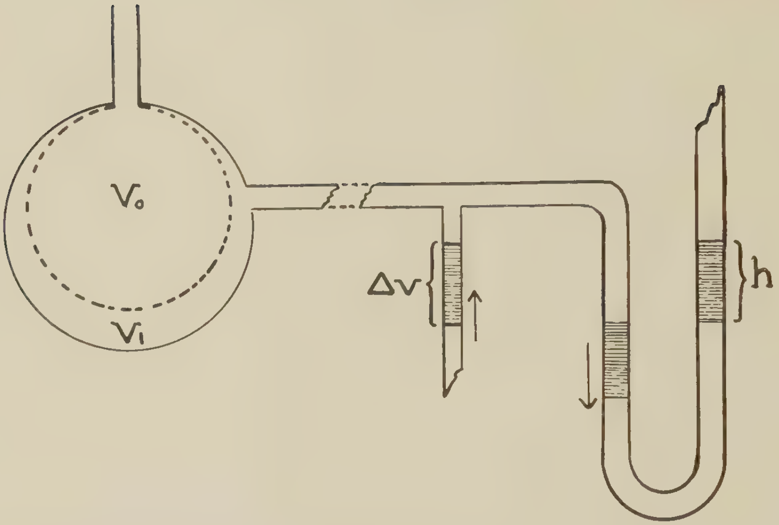

FIG. 2.
Diagrammatic sketch of the plethysmograph and the manometer showing the schematic method of calibrating the manometer.

A method of estimating the percentage changes of volume of an organ is outlined (Fig. 2). The apparatus is calibrated in the usual manner by noting the change in pressure recorded by the water manometer when the volume of the system is changed a known amount.

Let v = volume (cc.) of empty plethysmograph, including the tube leading to the U-tube.

v_0 = volume (cc.) of organ placed in plethysmograph.

v_1 = volume (cc.) of closed system.

Δv_x = decrease in volume (cc.) of closed system controlled by water or mercury in graduated burette.

h_x = increase in pressure (cm. of water) in system when volume is decreased Δv_x.

p = pressure (cm. of water) of atmosphere.

r = radius (cm.) of U-tube.

With the empty plethysmograph attached to the water manometer suppose a decrease in volume, Δv, increases the pressure h.

Then approximately,

$$p\,(\Delta v - \pi r^2 \frac{h}{2}) = vh, \text{ or}$$

$$v = \frac{p}{h}\,(\Delta v - \pi r^2 \frac{h}{2})$$

where $\pi r^2 \frac{h}{2}$ is the increase in volume in the U-tube due to decrease in volume Δv with consequent increase of pressure.

With the organ in the plethysmograph and attached to the water manometer, suppose a decrease in volume Δv_1 increases the pressure h. Then

$$(\Delta v_1 - \pi r^2 \frac{h}{2}) = v_1 h, \text{ or}$$

$$v_1 = \frac{p}{h}(\Delta v_1 - \pi r^2 \frac{h}{2})$$

Therefore the volume of the organ, v_0, is equal to

$$v_0 = v - v_1 = \frac{p}{h}(\Delta v - \Delta v_1)$$

In general, if the increase in volume of the organ causes an increase of pressure h_x, then Δv_x, the increase in volume of the organ, is given by

$$\Delta v_x = \frac{h_x}{h} . \Delta v_1$$

and the percentage increase in volume of the organ becomes

$$\frac{100 \Delta v_x}{v_0} = 100 \frac{h_x}{h} \frac{\Delta v_1}{v_0}$$

It is possible to use any liquid, such as alcohol, to increase the sensitivity, or to increase the specific gravity with sodium chloride, so as to indicate changes of pressure directly in units of 1000 dynes for each centimeter.

Summary: A photographic method of recording plethysmograms is described. The use of a U-tube liquid manometer, with its low inertia and low interval friction, furnishes a rapidly acting system for recording small intrinsic changes in pressure. These changes of pressure may be converted into changes in volume by the use of equations.

4474

Studies on Growth. Growth Factors in Liver.

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Recent investigations have emphasized the value of liver as a dietary supplement in diets apparently adequate with respect to vitamins A and D. No definite information is available, however, concerning the nature of the water soluble substances also present in liver.[1] This is a preliminary report of a study of the nutritional factors concerned with the growth of young white rats, and particularly of the presence of such growth factors in liver.

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[1] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxxii, 309; 1923-24, lviii, 363.

Young male and female rats, 4 to 5 weeks of age, were used. The basal diet was the fat-free diet of Evans and Burr[2] (purified casein, 25; commercial sucrose, 75; Osborne and Mendel salt mixture, 4). In every case this diet was supplemented daily with 9 drops of cod liver oil. The rats were kept in individual raised cages.

Rats placed upon this basal diet failed to survive. The addition of 0.15 gm. of dried yeast permitted survival but not growth. Growth occurred with the further addition of autoclaved liver or yeast. Rats fed the basal diet plus 0.5 gm. of dried liver daily grew

TABLE I.

Comparison of growth of rats on purified and unpurified diets.

Diet	No. of rats	Sex	Weight when 90 days of age		Weight when 120 days of age		Remarks
			Range in weight	Mean weight	Range in weight	Mean weight	
			gm.	gm.	gm.	gm.	
Mixed stock diet	12	♀	138-186	156	158-204	179	Increasing in wgt.
Basal diet plus 0.15 gm. yeast 0.50 gm. liver	17	♀	119-176	144	132-196	158	Maximum wgt.
Basal diet plus 0.5 to 1.0 gm. yeast	7	♀	112-152	132	137-170	149	” ”
Mixed stock diet	6	♂	182-274	211	210-342	260	Increasing in wgt.
Basal diet plus 0.15 gm. yeast 0.50 gm. liver	9	♂	162-194	173	164-240	206	Increasing in wgt.

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[2] Evans, H. M., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 741.

well for 20 to 30 days only. When this diet was supplemented with an extract containing vitamin B_1, prepared by the method of Seidell,[3] growth was resumed. Similar results were obtained using autoclaved liver. These experiments demonstrated that the limiting factor in liver was vitamin B_1 and that the liver was rich in vitamin B_2.

The purified diet supplemented with liver or yeast was lacking in some essential growth factor. Female rats on the mixed stock diet averaged 179 gm. in weight when 120 days of age and were still increasing in weight (Table I). Female rats on the purified diet reached an average maximum weight of 150 to 160 gm. after 90 days (when 120 days of age). Approximately the same results were obtained by supplementing the basal diet daily with 0.5 gm. of yeast, or with 1.0 gm. of yeast, or with 0.15 gm. of yeast plus 0.5 gm. of liver. These rats resumed growth when changed to the stock diet. The use of commercial casein has uniformly resulted in improved but subnormal growth. Our results differ from those of Evans and Burr,[2] who obtained normal growth by the addition of liver and yeast to the fat-free diet and also from those of Palmer and Kennedy,[4] who recently reported excellent growth following the addition of yeast alone to this same basal diet.

4475

Calcium and Phosphorus Studies in a Case of Clinical Hyperparathyroidism.

GENEVIEVE STEARNS AND J. D. BOYD.

From the Department of Pediatrics, State University of Iowa.

The subject of this study was a boy of 19, with progressing bowing of the legs, and other evidence of bone softening. On admission the serum calcium was 16.8, serum inorganic phosphorus 3.0 mg. per 100 cc. Notwithstanding a high calcium intake, the patient was losing about 0.5 gm. of calcium a day. The urinary calcium was 2 to 3 times the normal output while the fecal calcium nearly equalled the intake. There was a retention of phosphorus but the urinary phosphorus was above normal.

On the basis of the laboratory and roentgenological findings, a clinical diagnosis of hyperparathyroidism with resultant generalized

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[3] Seidell, A., *J. Am. Chem. Soc.*, 1922, xliv, 2042.

[4] Palmer, L. S., and Kennedy, C., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 427.

osteitis fibrosa[1, 2, 3, 4] was made. Exploration revealed an adenoma of the left lower parathyroid, which was removed. Just prior to operation, serum calcium was 17.5 mg., serum phosphorus 2.2 mg. per 100 cc.; 18 hours after operation the serum calcium had fallen to 12.3 mg. per 100 cc. It continued to fall, but more slowly, the values for the succeeding days being 10.7 and 8.5 mg. per 100 cc. respectively. The inorganic phosphorus of the serum remained low. From the second to the sixth day after operation a total of 6 cc. of parathyroid extract was given hypodermically.

Ten days after the removal of the adenoma, with the same diet as during the period of pre-operative observation, the patient was retaining about 0.25 gm. of calcium and 0.6 gm. of phosphorus a day. The urinary secretion of calcium had fallen to below normal values; the urinary phosphorus also was decreased, but to a lesser extent. At this time the serum calcium was 5.0 mg., phosphorus 3.0 mg. per 100 cc. Chvostek's sign became strongly positive the second day following operation and remained so for several days; no further signs of tetany were noted.

This study was made possible through the courtesy of the Department of Orthopedics.

4476

Dental Caries: Influence of Potential Mouth Acidity.

CATHERINE MAGEE, C. L. DRAIN AND J. D. BOYD.

From the Department of Pediatrics, State University of Iowa, Iowa City.

In a previous report, arrest of caries in childhood following the use of high vitamin, high mineral diets was described.[1] In order to determine whether such arrest might be credited to changes in the potential acid-producing power of the oral flora, bacteriological studies have been made before, during and at the completion of arrest of caries. Scrapings were taken from the gingival surfaces of teeth, and placed in tubes containing 3 cc. of isotonic saline; after suspension, 0.5 cc. portions were transferred to each of 3 tubes of

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[1] Mandl, F., *Arch. f. Klin. Chir.*, 1926, cxliii, 245.

[2] Gold, E., *Mitteilungen a. d. Grenzgeb. d. Med. u. Chir.*, 1928, xli, 63.

[3] Barr, D. P., Bulger, H. A., and Dixon, H. H., *J. Am. Med. Assn.*, 1929, xcii, 951.

[4] Stewart, C. P., and Percival, G. H., *Phys. Rev.*, 1928, vii, 283.

[1] Boyd, J. D., and Drain, C. L., *J. Am. Med. Assn.*, 1928, xc, 1867.

1% dextrose meat infusion broth, with pH of 5.2, 6.4 and 7.0, respectively. These were incubated for 48 hours at 37° C. The pH was then determined colorimetrically by comparison with standard tubes. The types of organisms present in the cultures were determined from smears stained by the Gram method.

Three children with active caries were studied. At the initial observation, the acid-producing power of the peridental flora was quite marked. The children were then placed on a diet such as is considered optimum for the normal child. Intake was insured by individual supervision, and additional foods were not allowed. With no further change in regime, the caries had become arrested in every case within less than 3 months. In no case was there significant change in the potential mouth acidity during the period of observation. A fourth child, with no dentinal caries but with decalcification of the gingival enamel, was included in the study. The same capacity of the mouth flora to produce acid was noted.

The data are offered as further evidence[2] that dental caries is not primarily dependent upon the production of acid in the mouth.

TABLE I.

Subject	Date	Initial pH of media			Remarks.
		5.2	6.4	7.0	
		After 48 hrs. at 37° C.			
L. H. age 11	1/19	4.8	4.6	4.8	Active caries
	1/31	4.8	4.4	4.6	Diet started
	2/12	4.8	4.8	4.4	
	2/16	5.0	4.6	4.6	
	3/2	4.2	4.4	4.6	
	3/16		4.2	4.2	Caries definitely arrested
	4/11	4.4			
	4/23	4.5			
E. B. age 10	1/19	4.8	4.6	4.8	Active caries
	1/31				Diet started
	2/12	4.6	4.4	4.4	
	2/16	5.0	4.6	4.6	
	3/2	4.6	4.4	4.6	
	3/16		4.2	4.2	Caries definitely arrested
T. W. age 6	1/31				
	2/12	4.6	4.6	4.4	Diet started
	2/16	5.0	4.8	4.8	
	3/2	4.2	4.2	4.4	
	3/16		4.2	4.6	
	4/11	4.4			
	4/23	4.5			Caries definitely arrested
V. G. age 14	4/5	4.6	4.8		No dental caries: decalcified enamel, diet started.
	4/7	4.8	4.6		
	4/10		4.4		
	4/12	4.6	4.4		

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[2] Roskin, M., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 465.

4477

Effect of Injections of Urine from Pregnant Women on Ovary of the Rabbit.

MAURICE H. FRIEDMAN. (Introduced by Samuel Goldschmidt.)

From the Department of Physiology, University of Pennsylvania.

In the opinion of Marshall, the fact that the rabbit does not ovulate spontaneously, but only after coitus, and then at a definite time (10 hours) afterwards, "points to the conclusion that the follicles discharge in response to a stimulus conveyed to the ovary by its nerves."[1] That the ovarian nerves are not indispensable is clear from the demonstration that ovulation may occur in the transplanted ovary.[2] Some humoral mechanism, therefore, may be safely assumed. The question arises, however, of the importance of this humoral mechanism. Is it, or is it not, a significant factor in the whole series of processes involved in ovulation in the normal rabbit? If it is of great significance, one should be able to provoke ovulation in the rabbit without coitus by injection of those substances which were responsible for the occurrence of ovulation in the transplants.

Stimulated by the recent work of Smith and Engle,[3] who were able to induce super-ovulation in the rat by the daily transplantation of fresh hypophyses, a series of rabbits were subjected to such transplantations, following exactly the technique of Smith and Engle. To date, it has been impossible to provoke ovulation in the rabbit by the transplantation of as many as 15 fresh rat hypophyses. Seeking for a more economical and more convenient source of the hormone of the anterior lobe, I was led to the use of urine from pregnant women by the reports of Zondek and Ascheim[4] in which it is stated that in such urine is found a substance whose biological effects closely simulate those of the anterior lobe of the hypophysis. In a series of female rabbits, in which a previous exploratory laparotomy had shown the presence of normal follicles and the absence of corpora lutea, the intraperitoneal injection of 12 cc. of urine from a pregnant woman, twice daily for 4 days, resulted in the appearance of fresh corpora lutea in the ovaries of each of the 7 rabbits so treated. Similar treatment of 9 rabbits with the urine of non-pregnant women was without effect.

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[1] Marshall, "The Physiology of Reproduction," Longmans, Green and Co., London, 1922, 561.

[2] Friedman, M. H., "On the Mechanism of Ovulation in the Rabbit," in press.

[3] Engle and Smith, *Am. J. Anat.*, 1927, xl, 159.

[4] Zondek and Ascheim, *Klin. Woch.*, 1927, vi, 248.

Histological study of these corpora lutea, however, show conclusively that ovulation did not occur, and that the corpora lutea produced by the injection of urine from pregnant women are the result of lutein transformation of corpora hemorrhagica with retained ova. Nevertheless, the clear differentiation between the effects of urine of pregnant women, and the urine of non-pregnant women, and the ease with which these effects can be seen by purely gross examination, justifies further study on a statistical basis to determine whether or not this procedure will be of clinical use in the diagnosis of pregnancy.

4478

Some Pharmacological Reactions of Benzothiazole.

MARSTON T. BOGERT, DAVID ANCHEL, AND HELEN G. HUSTED.
(Introduced by Israel S. Kleiner.)

From the Laboratory of Organic Chemistry of Columbia University, and the Laboratories of the New York Homeopathic Medical College and Flower Hospital.

Benzothiazole was prepared according to the method of Bogert and Stull,[1] by condensing o-amino thiophenol hydrochloride with formaldehyde. The constant boiling point of 231° C. corresponded with that given in the literature.

Owing to the fact that many compounds containing reduced or divalent sulfur are physiologically active, it seemed worth while to test the pharmacological action of this base. Accordingly, experiments of 2 types were performed; namely, cutaneous and intravenous tests.

In the cutaneous tests, a minute droplet of the oil was placed on the unbroken skin of the forearms of women and of men. Forty per cent of the number tested (43) developed a local dermatitis: the redness, swelling, and violent itching involving an area of at least 2 sq. cm. In the attempt to correlate these positive findings with various factors, it was observed that blondes seemed to be more susceptible than brunettes, but that sex apparently played no part. Two remarkable phenomena were noted; namely, delayed reactions, and increased hypersensitivity with repeated application. In most of the cases, the reaction appeared in from 2 to 18 days after the oil had been applied (average 9½ days), and in many it reappeared after having subsided for a few days. In one case, the application of the drug to the right wrist after the reaction on the left wrist had partly subsided caused, in addition to a direct reaction, a highly intensified recurrence of the original reaction of the left wrist.

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[1] Dissertation of Stull, 1925.

For the intravenous administration, the benzothiazole was emulsified in saline or in an autogenous serum. Injection into cats caused a marked fall in blood pressure, with an increase in the pulse rate. The effect on the respiration varied, depending on the dosage. A small dose sometimes caused an increase in both the rate and the amplitude, while larger doses usually had the opposite effect.

Approximately 100 mg. per kilo. of body weight, administered in a single dose, intravenously, constitute the minimum lethal dose for a cat. If this total amount be given in several small injections, it is not always fatal; but if divided into 2 or 3 large doses, it is usually lethal. In every animal, the respiration ceased a few minutes before the heart stopped beating.

4479

Effect of Respiratory Depressants on the Respiratory Quotient of Excised Tissue.

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From the Russell Sage Institute of Pathology in affiliation with the Second (Cornell) Medical Division of Bellevue Hospital.

We have investigated the effect of various depressants of the oxygen consumption of excised kidney tissue of the rat in the Warburg apparatus.

Table I gives the oxygen consumption and the respiratory quotients, and Table II the calculated effect on carbohydrate and fat using the Zuntz-Schumburg-Lusk table.

With the depression of oxygen consumption there was an elevation of quotient in 9 out of 11 observations. Analysis of these data indicates that the oxidation of fat is interfered with more than that of carbohydrate. This is in contrast to the situation in diabetes where the primary injury is to the oxidation of carbohydrate.

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* Most of these results were obtained in 1925-1926 under grants from the National Research Council and the Committee on Scientific Research of the American Medical Association.

TABLE I.
Respiratory Quotients after Depression of Oxygen Consumption.

Depressant	cmm. O_2/dry mg./hr.		Respiratory Quotient	
	Without depressant	With depressant	Without depressant	With depressant
Ethyl urethane 3%	19.3	13.5	.814	.922
” ”	19.3	10.6	.904	.885
” ”	22.7	11.8	.863	.934
” ”	15.2	9.2	.812	.883
” ”	17.5	13.0	.760	.833
Thymol .012%	22.8	13.0	.878	.929
” .014%	19.8	11.7	.872	.919
” .012%	20.2	17.4	.894	.919
” .010%	25.0	17.9	.908	.888
After previous anaerobic exposure	19.7	11.3	.894	.932
”	19.2	7.5	801	.863

TABLE II.
Selective Action of Depressants on Oxidation of Fat and Carbohydrate.

Depressant	No. of experiments	Average depression of O_2 cons.	Average effect on fat	Average effect on carbohydrate
		%	%	%
Ethyl urethane 3%	5	—38	—57	+7
Thymol .010 to .014%	4	—30	—52	—19
Anaerobiosis	2	—59	—68	—33

4480

Observations on the Pathogenesis of Pulmonary Suppuration in the Albino Rat.*

THEODORE S. MOISE AND ARTHUR H. SMITH.

From the Department of Surgery and the Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.

The well recognized syndrome of pulmonary suppuration in the albino rat is, as in many human affections, an advanced stage of the condition. The earlier manifestations of the disease are imperfectly understood and the common appellation "pneumonia" for the well developed lesion is a reflection of this lack of knowledge of the fundamental nature of the process. The lesions of the lungs vary greatly according to the stage and extent of the process but the

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* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

essential nature of the completely developed disease is that of chronic lung abscesses.

We have been particularly impressed with the frequent occurrence of atelectasis in white rats which are apparently in perfect health. These observations lead us to suggest that atelectasis subsequent to bronchial obstruction is a primary factor in the development of pulmonary infection in the white rat. To substantiate this hypothesis it is necessary to show that obstructive atelectasis occurs in white rats showing no evidence of pulmonary infection and that organisms may gain an entrance to the lungs and selectively localize in the collapsed lobes. The probable pathway of infection has been indicated by Jones,[1] who isolated organisms occurring in the environmental atmosphere, from the lungs of several laboratory animals. The regularity with which the cultures were positive varied directly with the amount of dust in the atmosphere. It is a well known fact that after bacteria have once gained entrance into the body their ability to proliferate and invade the host is greatly increased by an obstruction to the part.

Our own observations have clearly shown that an obstructive atelectasis is a very common finding in white rats. This condition has been observed many times in animals showing no gross or microscopic evidence of pulmonary infection. The atelectatic areas vary in size from a minute portion at the tip of a lobe to a massive collapse of the entire right side and the infra-cardiac lobe. In many instances we have demonstrated occlusive mucus plugs in the bronchi leading to collapsed lobes. The points of occlusion have been demonstrated by the injection of air and fluid into the trachea and by careful dissection of the bronchi leading to collapsed lobes. The objection that the occlusion may develop as the result of infection can be readily answered as we have frequently observed obstructive atelectasis in animals showing no evidence of pulmonary infection. If the obstruction persists, small abscesses are frequently seen within the collapsed portions of the lung. As the infection progresses the abscesses become larger and all evidence of a pre-existing atelectasis is obscured.

On the basis of these observations we suggest that the sequence of events in the pathogenesis of pulmonary infection in the white rat is an obstructive atelectasis followed by the growth of organisms and the development of pulmonary suppuration.

This report is based on incidental observations made during the course of experiments on the effect of dietary protein on the remaining kidneys of rats after a unilateral nephrectomy. More than 200 animals have been studied and the lesions described have occurred independently of the diets fed.

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[1] Jones, F. S., *J. Exp. Med.*, 1922, xxxvi, 317.

4481

Relation of Vitamins B (B_1, F) and G (B_2) to Renal Enlargement in Rats Fed High Concentrations of Protein.*

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From the Laboratory of Physiological Chemistry and the Department of Surgery, Yale University, New Haven, Conn.

That vitamins B and G play a unique and important rôle in the metabolism of proteins is believed by some investigators. Reader and Drummond[1] have stated that there is little hypertrophy of the kidneys of rats fed on diets rich both in protein and in vitamin B complex, and that the required vitamin B complex is quantitatively related to the amount of protein in the diet. As this conclusion was not in accord with certain of our own observations we planned experiments to test the thesis that renal enlargement produced by high protein diets is lessened by increased ingestion of vitamins B, G and the vitamin B complex.

Groups of 12 to 14 animals (30 days old), consisting of intact rats as well as those from which one kidney had been removed, were fed diets of 18 and 90% protein respectively for 56 days. The groups were fed 3 different levels of yeast and in addition the highest level of yeast was supplemented with (1) factor G, using autoclaved yeast or (2) factor B, in the form of tikitiki extract.† Additional experiments are in progress to test the effect of an excess of the heat-stable (vitamin G) and heat-labile (vitamin B) factors fed separately without yeast. At autopsy hearts and kidneys were weighed and renal enlargement calculated on the basis of the weights of these organs as published by Donaldson.[2]

In the accompanying table are presented experimental data which show that: 1. Renal enlargement occurs in both nephrectomized

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* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Reader, V., and Drummond, J. C., *Biochem. J.*, 1926, xx, 1256.

† Furnished through the kindness of Dr. Brown of the Philippine Bureau of Science.

[2] Donaldson, H. H., "The Rat," 1924, 2d ed., Philadelphia, Penn.

TABLE I.
Kidney Enlargement on High Protein Diets Fed with Varying Amounts of Vitamins B and G.

	Protein in diet	Yeast (gm.)				
		0.4	0.8	1.6	1.6+ 3 drops tiki-tiki extract	1.6+ 7% autoclaved yeast
	%	Enlargement (%)				
Nephrectomized rats	18	57	52	64	60	51
	90	115	119	124	127	129
Difference		58	67	60	67	78
Intact rats	18	-7		4		
	90	39		50		
Difference		46		46		

and intact rats fed high protein diets. 2. This enlargement takes place irrespective of the amount of yeast consumed. 3. Supplementing yeast with additional vitamin G or vitamin B does not lessen the enlargement. 4. The consistent enlargement found does not indicate any quantitative relationship between the intake of protein and vitamins B and G in reducing renal hypertrophy as suggested by Reader and Drummond.

4482

Correlation in Development of Structures Associated with Transplanted Eyes.

VICTOR C. TWITTY. (Introduced by Ross G. Harrison.)

From the Osborn Zoological Laboratory, Yale University.

Eyes were transplanted in normal position between embryos (tailbud stage) of *Amblystoma punctatum* and *A. tigrinum*, forms of contrasting size, and the animals reared until the approach of metamorphosis. The grafted organs retain their specific size on the foreign species, and are hence out of scale with the associated structures of the host. In response, the latter exhibits certain morphological adjustments, particularly in the brain, eye-muscles, and chondrocranium.

When the nerve of the large transplanted *tigrinum* eye makes normal connection with the brain of *punctatum*, the smaller species, there follows a hyperplasia of 10-20% in the gray matter of the opposite half of the midbrain, while in the reciprocal combination, or with one eye totally extirpated, there is a corresponding hypoplasia of 5-10%. In the diencephalic optic centers changes in cell-proliferation are relatively unimportant, and often lacking. In the region of the chiasma (preoptic nucleus), however, there are almost invariably strong hyper- or hypoplastic changes on the same side as the grafted eye. This suggests the possibility that there is normally a homolateral innervation of this region by fibers from the retina, contrary to general belief. In animals with grafted *tigrinum* eyes the gray matter in the dorsal part of the diencephalon opposite the transplant shows a tendency, often quite pronounced, to shift ventrally as if to meet the ascending optic tracts from the large eye. It seems probable that the visual fibers exert an attractive influence on the cells of this optic center.

The eye-muscles, developing from the host, adjust their size in the direction indicated by the size of the grafted eye, although they never attain the size characteristic for the normal muscles of the eye in question. The hypertrophy or atrophy, as the case may be, is accomplished by changes in the number, rather than the size, of the individual fibers. The hyperplasia of fibers in the *punctatum* muscles developing upon a grafted *tigrinum* eye was found to average about 50%.

The cartilaginous eye-capsule, which develops extrinsically, generally assumes the size characteristic for the transplanted eye, rather than for the normal eye of the host. The size of the trabecula, which forms the lateral wall of the brain-case in the orbital region, is also correlated with the size of the adjacent transplanted eye. The *punctatum* trabecula next a *tigrinum* eye shows a very marked enlargement throughout most of its length. When the nasal placode is grafted also, the effect extends to all elements of the capsule of the nose.

4483

Seasonal Variations in the Interstitial Tissue of the Testis of the Horned Toad (Phrynosoma solare).

RAYMOND F. BLOUNT. (Introduced by J. S. Nicholas.)

From the University of Arizona and the Osborn Zoological Laboratory, Yale University.

A histological study has been made of a series of Phrynosoma testes taken at frequent intervals throughout the year, with special reference to the problem of interstitial cell function. If, as thought by some, their function is the control of sex activity then they should show seasonal changes correlated with the breeding period. Although such work has been extensive on other groups, particularly in the amphibia, practically nothing has been done on the reptiles.

Seasonal changes in the interstitial tissue should be expressed quantitatively. In this it is important to differentiate between the volume of tissue and the number of cells since these do not seem to be directly proportional. In expressing volume changes, the actual volume and not relative volume was calculated since the testis as a whole shows seasonal variations in size. Counts of the number of interstitial cells per unit volume were made and from these the relative number of cells for testes of different seasons was calculated.

The results show definite testicular cycles. The total volume of the testis undergoes a marked seasonal change, reaching a maximum during the breeding season. This is an external expression of the spermatogenetic cycle which gives changes in diameter, total length, and volume of the seminiferous tubules. These changes have been measured and recorded quantitatively.

The interstitial cell cycle is definitely correlated with the spermatogenetic cycle. The size of the interstitial cells and of their nuclei is greatest during the breeding season. Following the period of sex activity a syncytial condition exists in which the cell boundaries are very indistinct or totally lacking. At this time the nuclei are smallest. At no time do these cells revert to a connective-tissue-cell type. The number of interstitial cells decreases as the breeding season advances. The minimum number is reached at a time when the testis is gorged with spermatozoa. There is a marked increase in the number of interstitial cells at the close of the breeding season when all the spermatozoa are extruded. The volume of interstitial tissue is twice as great during the height of sperm production as it is during the period which follows.

The ovary undergoes similar volume changes which culminate in ovulation. These changes are correlated in time with the conditions occurring in the testis and with the time of observed copulation.

The fact that the number of interstitial cells is at a minimum during the breeding season has led some workers to the conclusion that the interstitial cells have no endocrine function. This has been decided without work of a quantitative nature and it has not been realized that the volume of interstitial tissue may be greatest during the breeding season. The present work would indicate that if the interstitial tissue is responsible for sex characters and behavior, it is the volume of tissue and not the number of cells that is important.

4484

Movements in Transplanted Limbs.

J. S. NICHOLAS.

From the Osborn Zoological Laboratory, Yale University.

In the course of the experimental work involving the use of the amphibian limb disc, many of the effects of this developing structure upon the nervous system have been discovered. Most of the transplantations have involved placing the limb disc posterior to its normal location. Such transplants show very weak function unless they are innervated by at least one of the nerves which under ordinary conditions would go into the normal limb. Detwiler and Carpenter[1] have studied coordinate movements in both the transplanted and normal limbs and have found that coordination is an expression of an innervation common to both the normal and the transplanted limb through some one of the bronchial plexus nerves. Such coordination is lost as soon as the communicating branches of such nerves are cut.

The work here reported deals with transplantations made in testing the growth of various isolated parts of the amphibian nervous system. The operation, in which the limb and associated tissues were used in order to block the growth of the nervous system, involves the removal of a part of the nervous system and the transplantation into the wound of a rectangular transplant of embryonic tissue which includes the rudiment of the limb and also of the pronephros. The establishment of a block within the nervous system has been reported previously.[2]

The transplants have been located either anterior or posterior to the normal limb level and their reactions noted. Their regional locations are (1) above the notocord in the region formerly occupied by the ninth, tenth and eleventh spinal cord segments; (2) in the region formerly occupied by the tenth cranial, the first and second spinal segments; (3) in the region of the mesencephalon and (4) in the orbit.

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[1] Detwiler, S. R., and Carpenter, R. L., *J. Comp. Neur.*, 1929, xlvii, 427.

[2] Nicholas, J. S., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 662.

These transplantations because of the variety of the regions occupied by the developing limb afforded an opportunity for the study of the functions of limbs with innervations far different from the normal, both in their source and in the composition of their components.

The limbs transplanted into the region of the ninth, tenth and eleventh spinal segments are seldom functional although they are in direct relation with the cut end of the spinal cord from which fibers grow into the limb. The nerves which penetrate the limb are derived directly from the spinal cord or from the posterior branches of the spinal nerves and are found in normal pattern relationships within the limb. Quantitatively, the limbs are well innervated but the nerves do not possess the qualitative action necessary to produce physiologically complete muscular movements within the limb.

The limbs situated in the region of the tenth cranial, first and second spinal segments give some degree of function in 80% of the cases. Of these, 40% show movements which may be defined as normal, *i. e.,* the limb is capable of flexion, extension, abduction and adduction of either the whole limb or its parts. In the 20% nonfunctional limbs, the physiological deficiency was not due to failure of nerves to grow into the limb but to incoordinate action produced by aberrant nervous connections. These produce a simultaneous contraction of all the muscles in the limb resulting in a condition of dominant extension tonus or spasticity. The limbs which are termed functional are not coordinate with the normal limbs in their movements but are coordinate with muscle groups supplied by similar nerve groupings. The limbs transplanted to the medulla region showed movements which were coordinate with gill movements.

The innervation to such transplants may arise either through the seventh or ninth cranial nerves or through nerve cables which arise directly from the medulla and have no anatomical peripheral connections with cranial or spinal nerves. The reactions of the limb are physiologically similar whether the limb is innervated by a peripheral trunk such as the seventh or ninth cranial or by a direct connection with the medulla.

When limbs are transplanted in place of the mesencephalon, they may be innervated by the fifth or seventh cranial nerves. These transplants do not develop limbs in nearly so high a percentage of cases as in the preceding series due to failures in the vascular supply. When, however, they do develop they show the same conditions of function as those given above.

When the limb bud is transplanted into the orbit in place of the *globus oculi,* very few fully formed limbs develop. This location.

however, affords a chance to study the movements of the limb on the one side and to compare it with the normal movements of the eye upon the opposite side. So far in the course of experimentation only one limb has been secured which has shown perfect development in this location. This limb likewise showed movements of the whole limb which corresponded to the movements of the normal eye. The limb did not show movements of its component parts.

The experiments show that when the limb disc is transplanted to regions anterior to its normal location, functional connections can be effected through cranial nerves or through fiber outgrowths direct from the nervous system. Cranial nerve components which under ordinary conditions are associated with the somatic motor, special visceral (splanchnic) motor and sensory regions are capable of producing reactions within an extremity normally supplied by general motor and sensory elements. When such reactions are obtained they are coordinate with the reactions in groups supplied by the same innervation.

4485

Transplantations of Tissues in Fetal Rats.

J. S. NICHOLAS.

From the Osborn Zoological Laboratory, Yale University.

Transplantations have been used in order to determine the amount of differentiation which may take place in fetal mammalian structures and also to study the changes which may occur in such tissues following their transplantation to strange environments.

The method of procedure in operations upon the mammalian fetus has been described previously.[1] It is necessary to use fetuses of the earliest possible stage of development in order to secure the tissues in the least differentiated stages. This is difficult in the albino rat fetus because of the absence of anatomical landmarks which will assist the operator in localizing the tissue which it is desired to remove from the embryo. In order to secure an anatomical landmark a cross was made between albino and hooded stock. This cross gave both albino and hooded fetuses in the same litter, ofttimes within the same uterine horn. Pigment develops in the eye of the hooded rat at about the tenth day of gestation age. Unless some factor is used for orientation so much handling is necessary that the damage to the tissues precludes any chance of a successful experiment.

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[1] Nicholas, J. S., *Anat. Rec.*, 1925, xxxi, 385.

Two series of transplantations were performed: (1) transplantations of the *globus oculi* and (2) transplantations of the forelimb.

The *globus oculi* was removed from one animal of a litter and transplanted to various regions of a second fetus. A pigmented globus was used as the graft, the host being albino. In this way an accurate check and a simple method of localizing the transplant in the host were obtained. The eye was transplanted under the skin over the abdomen, within the abdominal cavity and under the skin of the thorax. Of these locations, the flank over the abdomen gave the greatest number of successful cases, the abdominal cavity next and the thoracic subcutaneous region the lowest.

Growth of the eye was secured in a relatively small percentage of the total number of experiments. Many of the eyes were so badly injured during the operations that successful results could hardly be expected. When the tissues are injured in this way, the eye acts as a foreign body and the host tissues give a typical foreign body reaction.[2] Where optimum conditions were secured at the time of transplantation, the graft was well vascularized and the tissues grew. The final increase in the size of the eye could be measured by comparing the ratio of the measurements of the graft and the normal eye of the host. In such cases, the transplant grew to 75% of its normal volume.

In the second series of experiments, the transplantation of the forelimb was attempted. This operation cannot be conducted with the same degree of certainty that obtains in the preceding series. The tissues are extremely fragile and must be handled with extreme delicacy, all cuts being made with a thin glass pipette in which the tissues are handled during their transplantation. In the light of the experiments with the eye, the region of transplantation was the skin of the flank over the abdomen.

Two successful transplantations were secured from 40 experiments. In one case, the limb was transplanted on the eleventh day, in the other on the thirteenth day of the gestation period. Both limbs showed relatively little differentiation beyond that at the time of transplantation. Vascularization was irregular and much less abundant than in the eye transplants. Some cartilage was formed and the skin layers were decidedly thickened over the transplant. All the remaining cases showed either complete resorption of the graft or else its loss by tearing during parturition.

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[2] Nicholas, J. S., *Anat. Rec.*, 1929, xlii, 58.

4486

A Test for the Mechanical Fragility of Blood.

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Oxalated venous blood is centrifuged and the plasma separated from the cells. Two mixtures are prepared, one consisting of 2 cc. of packed cells and 4 cc. of 0.9% NaCl solution, the other of 2 cc. of packed cells and 4 cc. of the plasma which had just been removed from the cells. A portion of each mixture is then placed in a glass stoppered vial of 3 cc. capacity. Each vial contains a segment of glass rod 5 mm. in diameter of such length that the weight is 0.6 gm. The vials are completely filled, no air being allowed to remain under the stopper. They are then shaken in a shaking machine 40,000 times at the rate of 560 per minute. The vials are then centrifuged and the supernatant fluid from each vial is separated from the cells. One cc. of supernatant fluid from each vial is mixed with 4.5 cc. of 1% NH_4OH solution. Illuminating gas is bubbled through these mixtures to turn the hemoglobin to carbon monoxide hemoglobin. Standards are prepared as follows: From that portion of the saline cell mixture which was not used to fill the vial a number of fills of a 20 cu. mm. pipette are taken into 5.5 cc. of dilute ammonia, until a rough match of color is obtained. After saturation with illuminating gas this standard mixture is placed in a colorimeter and the mixture of saline supernatant fluid and ammonia is read against it. Similarly a mixture is made of 1 cc. of plasma saved from the original centrifuge tube and 4.5 cc. of dilute ammonia. A number of 20 cu. mm. quantities of the plasma mixture left over after the vial was filled is added to this until a rough match is obtained with the mixture of supernatant plasma and ammonia. Then after passing illuminating gas through this standard the 2 solutions are placed in the colorimeter and a reading made. The amount of hemoglobin in the original saline cell and plasma cell mixture is determined by the Newcomer method. From these various readings it is possible to calculate the amount of hemoglobin set free in the supernatant fluid of each vial by the process of shaking and so to calculate the percentage of breakage.

In normal blood under the conditions listed above the average breakage in the saline tube is about 0.7%. The plasma tube shows a somewhat smaller amount of breakage, averaging 0.55%. Abnormal bloods have been observed which show a breakage as high as

4.0%. The results obtained are compared with those of the ordinary saline fragility test. Some interesting differences in the results of the 2 tests have been observed. For example, certain severe untreated cases of pernicious anemia have shown a definitely increased mechanical fragility at a time when the saline fragility test was normal. The clinical results of the test will be reported in full at a later date.